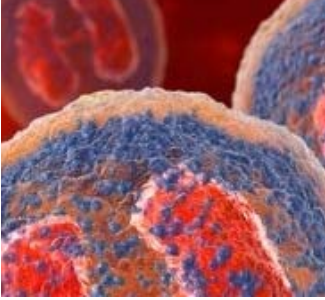


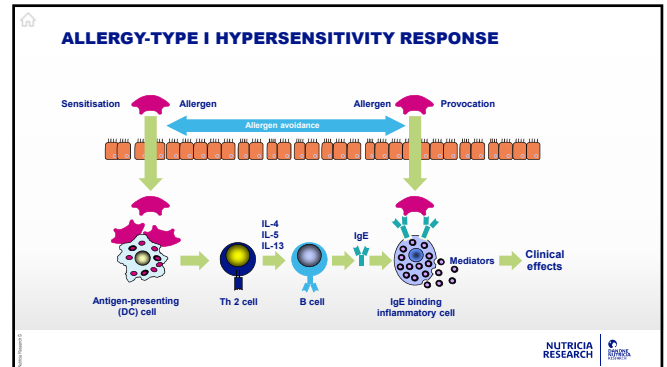
ALLERGENICITY ASSESSMENT

of cow's milk based hydrolysed infant formula

Prescilla Jeurink, PhD
TIFN & AgriFoodTop, 01 June 2016




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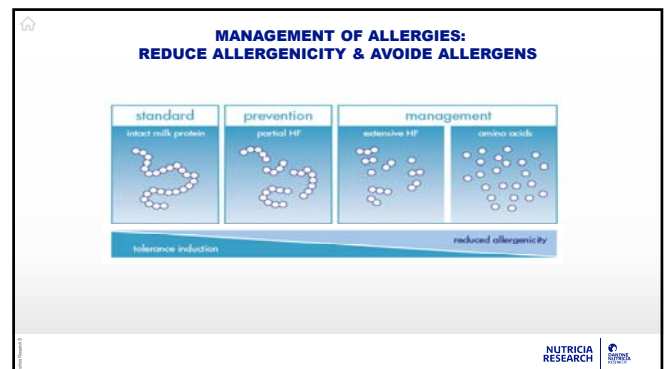


COW'S MILK ALLERGY

- Prevalence**
 - 2-3% of infants
 - Virtually all in the first year of life
- Prognosis/Tolerance**
 - 1 year – 50%
 - 3 years – 85%
- Clinical disorders**
 - Gastrointestinal: gastrointestinal anaphylaxis, allergic eosinophilic esophagitis and gastroenteritis
 - Skin: urticaria, angioedema, morbilliform rash, atopic dermatitis
 - Respiratory: rhinoconjunctivitis, acute bronchospasm vs. asthma
 - Systemic: anaphylactic shock



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LEGISLATION HYDROLYSED FORMULAS

According to the European food law, infant formulas should have an amount of **less than 1 % immunoreactive protein** in the formulas, **measured with methods generally acceptable as appropriate**.

The formulas **administered orally should not induce sensitization, in animals**, to the intact proteins from which the formulae are derived.

To guarantee the hypoallergenicity of eHF products, it should be proven that **at least 90% of cow's milk allergic children tolerate** the product. [1].

1. Commission Directive 96/4/EC of 16th February 1996 amending Directive 91/321/EEC on infant formulae and follow-on formulae. Official Journal of the European Communities, 1996, No L 49, p. 12-16.

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EVALUATION OF THE RESIDUAL ALLERGENICITY OF HYDROLYSED FORMULAS

Toxicology Letters 201 (2011) 264–268

Contents lists available at ScienceDirect

Toxicology Letters

Journal homepage: www.elsevier.com/locate/toxlet

In vivo and in vitro evaluation of the residual allergenicity of partially hydrolysed infant formulas

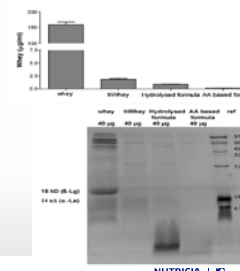
Betty C.A.M. van Esch^{a,b,c,1}, Karen Knipping^{a,b,1}, Prescilla Jeurink^{a,b}, Sicco van der Heide^c, Anthony E.J. Dubois^d, Linette E.M. Willemsen^a, Johan Garssen^{a,b}, Léon M.J. Knippels^{a,b}

^a Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands
^b Diverse Research – Centre for Specialized Nutrition, Wageningen, The Netherlands
^c Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, The Netherlands
^d Department of Pediatric Pulmonology and Pediatric Allergy, University Medical Center Groningen, University of Groningen, The Netherlands

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IN VITRO EVALUATION OF THE RESIDUAL ALLERGENICITY

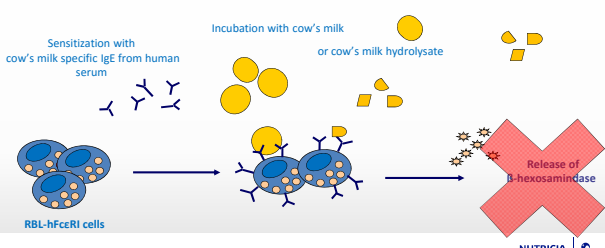
- Whey/β-lactoglobulin (BLG) ELISA**
 - Measurement concentration residual proteins
- SDS-PAGE / Immunoblotting**
 - SDS-PAGE: evaluation residual protein pattern
 - Immunoblotting: assessment binding specific antibodies to protein(fragments)
- Gel permeation chromatography**
 - Peptide size distribution



Van Esch, Knipping et al., Toxicology Letters, 2011

IN VITRO EVALUATION: THE RBL-ASSAY

- Rat basophil-like leukemia cells (RBL)-assay**
 - Mimic challenge NOT sensitization phase

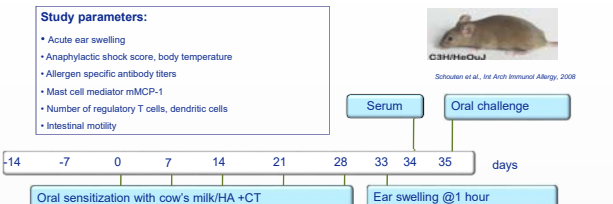


Van Esch, Knipping et al., Toxicology Letters, 2011

MOUSE MODEL FOR COW'S MILK ALLERGY

Study parameters:

- Acute ear swelling
- Anaphylactic shock score, body temperature
- Allergen specific antibody titers
- Mast cell mediator mMCP-1
- Number of regulatory T cells, dendritic cells
- Intestinal motility



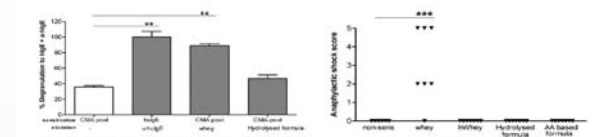
Disadvantage guinea pig model:

- High discomfort level
- Guinea pigs generate anaphylactic IgG1a instead of IgE antibodies.
- Predictive value of the guinea pig model questionable with regard to the extrapolation to the human situation.

Schouten et al., Int Arch Immunol Allergy, 2008

Van Esch, Knipping et al., Toxicology Letters, 2011

COMPARISON ALLERGIC REACTION IN VITRO VERSUS IN VIVO



Van Esch, Knipping et al., Toxicology Letters, 2011


VALIDATION OF MOUSE MODEL FOR ALLERGENICITY ASSESSMENT OF HYDROLYSED FORMULAS

Toxicology Letters 220 (2013) 95–102

Contents lists available at ScienceDirect

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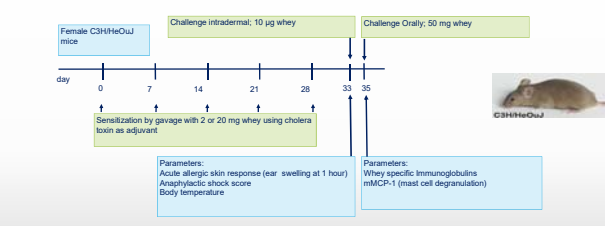
Interlaboratory evaluation of a cow's milk allergy mouse model to assess the allergenicity of hydrolysed cow's milk based infant formulas

B.C.A.M. van Esch^{a,*}, J.H.M. van Bilsen^b, P.V. Jeurink^c, J. Garssen^a, A.H. Penninks^d, J.J. Smit^a, R.H.H. Pieters^e, L.M.J. Knippels^c

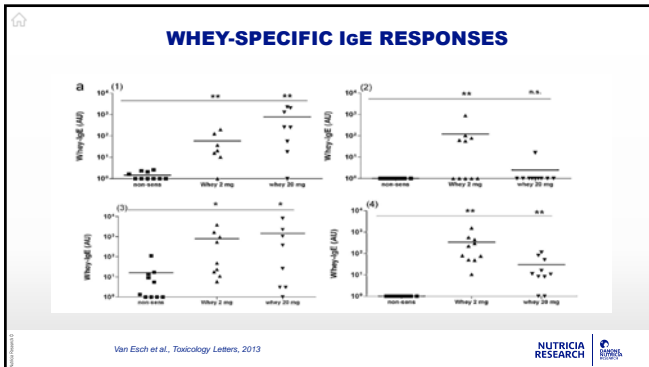
^a Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands
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Van Esch et al., Toxicology Letters, 2013

TRANSFER OF MOUSE MODEL FOR CMA TO 4 DIFFERENT RESEARCH CENTERS



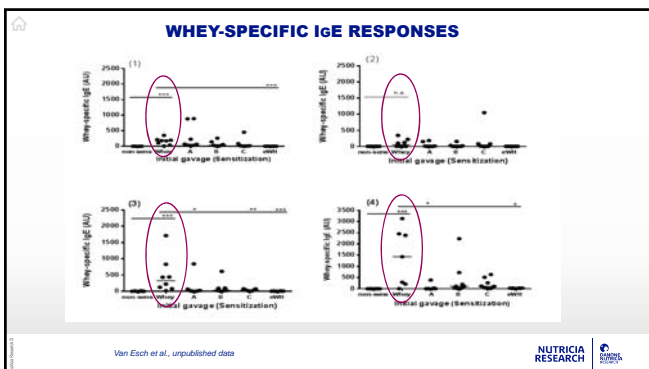
Van Esch et al., Toxicology Letters, 2013



SENSITIZING CAPACITY OF DIFFERENT HYDROLYSATES TESTED

- The sensitizing properties of three partially hydrolyzed whey proteins (pWHA, -B and -C) were included
- pWH are also tested in the guinea pig (model of reference; ASA test)
 - pWH-A Best results (6/0)
 - pWH-B failed test (2/0;3/3;1/5)
 - pWH-C failed test (3/0;2/3;1/4)

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CONCLUSIONS

- Despite the presence of whey-IgG1 (indicating exposure to whey), center 2 did not show elevated levels of whey-IgE. Therefore all results of this center are excluded
- Individual pWH treated mice did show (minor) allergic symptoms. No allergic symptoms were observed to eWH
- None of the centers were able to differentiate between sensitizations on all clinical parameters. Multiple clinical read-out parameters in addition to IgE is indispensable to predict allergenicity of hydrolysed whey proteins
- Significance of the individual parameters needs to be evaluated

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DISADVANTAGES/LIMITATIONS OF RBL-ASSAY WITH HUMAN SERUM

- Cow's milk allergic serum very difficult to obtain
- Serum mainly from cow's milk allergic infants (small volumes)
- Not all high cow's milk specific IgE binds to the RBL-cells
- High background degranulation possibly due to intervening substances
- Pooled serum needed; every new pooled serum needs to be validated against the previous

OPEN ACCESS Freely available online

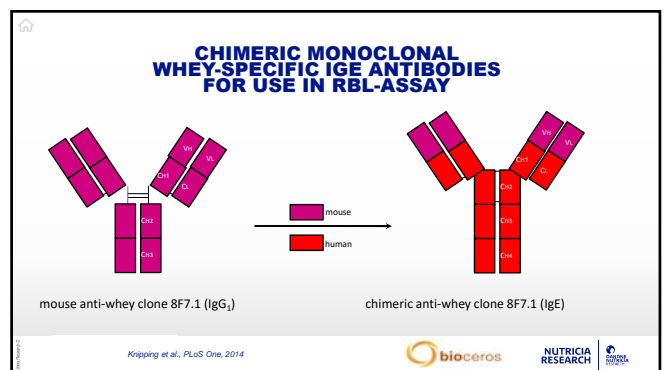
PLOS ONE

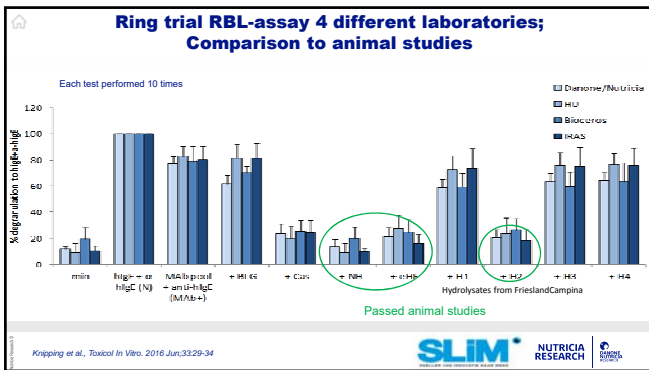
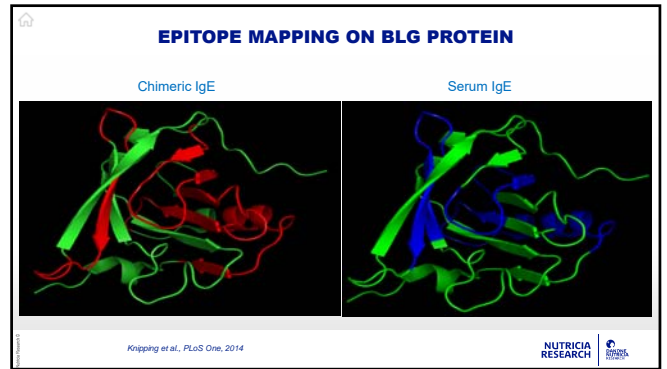
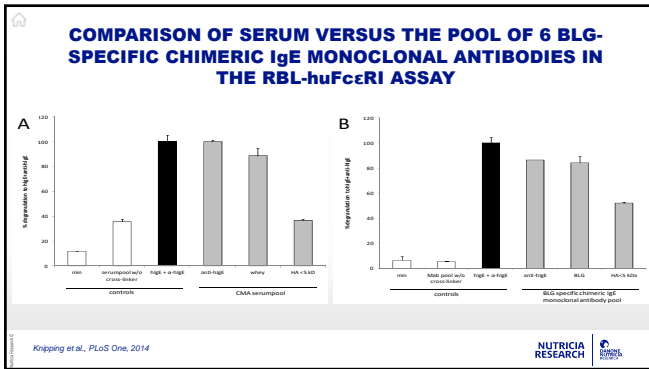
Development of β -Lactoglobulin-Specific Chimeric Human IgE κ Monoclonal Antibodies for *In Vitro* Safety Assessment of Whey Hydrolysates

Karen Krüpping^{1,2,3*}, Peter J. Simons^{4,5}, Laura S. Buisson-Sieumer⁶, Linda Cox⁶, Marcel den Hartog⁷, Debiao de Jong⁸, Reiko Takahara⁹, Johan Garssen¹⁰, Louis Boon¹¹, Leon M. J. Knippenberg¹²

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PROPOSAL ALLERGENICITY ASSESSMENT OF HYDROLYSED COW'S MILK BASED INFANT FORMULAS

Partially hydrolysed formula
(For children at risk of developing CMA)

In vitro

- Measurement residual whey proteins ELISA (immunoreactive proteins < 1%)
- Degranulation of RBL-huFcεRI cells (needed?)
- SDS-PAGE/GPC + Western Blotting (characterization product)

In vivo (sensitizing capacity)

- Mouse model for CMA
 - Oral sensitization with pWH and challenge with whey

Extensively hydrolysed formula
(For CMA infants)

In vitro

- Measurement residual whey/casein proteins ELISA (immunoreactive proteins < 1%)
- Degranulation of RBL-huFcεRI cells (Reduction/replace animal model)
- SDS-PAGE/GPC + Western Blotting (characterization product)

In vivo (allergenicity assessment)

- Mouse model for CMA
 - Oral sensitization with whey and challenge with eWH
- CT in CMA infants
 - At least 90% should tolerate product

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