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Correlation of the allergenicity and tolerogenicity of two cow's milk protein products with their intestinal uptake – a study in Brown Norway (BN) rats

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Proceedings of the 3rd International ImpARAS Conference



*This conference is organized by COST Action FA1402 ImpARAS
Improving Allergy Risk Assessment Strategy for new food proteins*

www.imparas.eu

Sponsors & organizers



Scope and welcome address

As chair of COST Action ImpARAS FA1402, it is a real pleasure to welcome you at our third ImpARAS conference in Helsingør in Denmark. ImpARAS, **Improving Allergenicity Risk Assessment Strategy** (for novel and modified proteins), is an European network that aims to build an interdisciplinary European network of scientists with a broad range of expertise to discuss, with an out-of-the-box view, new ideas and more predictive models and approaches to improve the current allergenicity risk assessment strategy. ImpARAS is helping to develop an improved allergenicity risk assessment strategy for novel proteins, by adding more predictive tools to the current risk assessment strategy, and accelerate the introduction of novel protein (sources) onto the market, to mitigate the concern of consumers around novel or genetically modified protein (products) and to advice policy makers on the safety of novel protein (products).

The networks focusses on different topics:

- Physical/chemical properties of proteins impacting allergenicity, including effect of processing, matrix effect, glycosylation, lipid binding, digestion, bioinformatics, protein purifications and analysis and others.
- In vitro methods to predict sensitization to food allergy, including epithelial transport of proteins, DC-T cell interactions, activation innate and adaptive immune system and others.
- In vivo methods to predict sensitization to food allergens, including mouse, rat or other models to measure effect on the immune system and others.
- Allergenicity Risk assessment, including current status, examples and applications, visions and others.

ImpARAS is a COST Action that is supported for 4 years (December 2014 – December 2018) by COST (European Cooperation in Science and Technology). More than 230 scientist from Industry, Universities, knowledge centers and regulatory bodies from 32 countries are united in ImpARAS. The network is active through a range of networking tools, such as meetings, workshops, conferences, training schools, and exchange of staff between partners also called short-term scientific missions (STSMs). ImpARAS is open to researchers from universities, public and private research institutions, as well as to NGOs, industry and SMEs. So you are welcome to join our network! You can find more information on our ImpARAS website; www.ImpARAS.eu or become member of our LinkedIn group.

I would like to take this opportunity to thank the local organizers for all their efforts to make this conference a success and to welcome you in the wonderful atmosphere of Denmark. Also thanks to the scientific committee that made its best to offer you such an exciting and interesting program.

I hope that you will enjoy the conference, make new contacts and start new opportunities with colleagues from other countries. Of course I also hope to meet you again on one of our future meetings.



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Scientific and organizing committees

Scientific committee

Kitty Verhoeckx

TNO, The Netherlands,
(Chair)



René Crevel

RENE CREVEL Consulting Limited, UK,
(Vice Chair)



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Denmark



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National Food Institute,
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TNO, The Netherlands



Astrid Kruizinga

TNO, The Netherlands



Marloes van der Wal-Bellaart

TNO, The Netherlands



Scientific Program

Day 1 - Tuesday October 10th

- 12:30-14:00 Registration
14:10-14:20 Opening welcome and practicalities, Charlotte B. Madsen, DTU
14:20-14:30 Presentation of COST Action FA1402 ImpARAS
Kitty Verhoeckx, Chair of the Action, TNO, The Netherlands

Chair: Erwin Roggen & Linette Willemsen

- 14:30-14:50 **Jolanda van Bilsen** - *A Bayesian network-based approach for discovering biomarkers for oral immunotherapy of food allergy.*
14:50-15:10 **Clélia Villemin** - *Characterization of reference proteins: native and modified gliadins for the Selection of in vitro model of allergenic risk assessment.*
15:10-15:30 **Simone Hayen** - *Combined exposure of intestinal epithelial cells to dietary oligosaccharide mixture scFOS/lcFOS and CpG DNA effectively enhances the Th1 and regulatory IL-10 response in a peanut-specific co-culture model.*
15:30-15:50 **Robin Gradin** - *Prediction of food protein allergenicity using the GARD assay*
15:50-16:20 Coffee Break
16:20-17:05 **Keynote lecture: Maria Rescigno** “*Host-microbe interactions in the gut*”

Chair: Katrine Bøgh & Kitty Verhoeckx

- 17:05-18:15 Flash presentations of ESRs on STSM (5 min + 1 min questions)
Joana Costa - Generation and characterization of a recombinant nonallergenic chicken tropomyosin as a potential molecular reference for allergenicity assessment studies.
Luigia Di Stasio - Comparison of the digestibility and antigenicity of raw and roasted whole peanut allergens.
Katrine Graversen - The effect of Akkermansia muciniphilia on house dust mite induced allergic airway inflammation
Tanja Kalic - Investigation of clinical and immunological reactivity of the major fish allergens parvalbumins.
Laure Castan - Food allergy skin sensitization: A comparative study with three different gluten products in Brown Norway rats.
Denise Schrama - Allergen identification in two main Mediterranean fish species using proteomics
Cristian Piras - Immunoproteomics characterization of allergenic and non-allergenic tropomyosin orthologs.
Caterina Villa - Lupine allergens in food products: a new real-time PCR approach for its detection and quantification.
Isabel Segura Gil - Detection of soy (Glycine max) allergens in processed model and commercial foods by ELISA techniques: Influence of selected target protein and ELISA format applied.
18:15-19:00 MC-meeting
19:00-21:00 Dinner

Day 2 - Wednesday October 11th

08:45-10:00 Working group 1-2-3-4 meeting

Chair: Gabriel Mazzucchelli & Clare Mills

10:00-10:45 **Keynote lecture: Michael Perkin** "Have EAT & LEAP left us any clearer as to how we should introduce allergenic foods to infants in real life?"

10:45-11:15 Coffee Break

11:15-11:35 **Marija Gavrović-Jankulović** - *Mass spectrometry based proteomic identification of extracellular proteins cleaved by protease activity of actinidin, a major allergen from kiwifruit.*

11:35-11:55 **Christof van Poucke** - *Detecting allergens in processed foods: the development of a multi-allergen LC-MS/MS method for baked goods.*

11:55-12:15 **Pasquale Ferranti** - *Production, digestibility and allergenicity of hemp (Cannabis sativa L.) based ingredients and products.*

12:15-12:35 **Ivan Dimitrov** - *Peptide fingerprints in food allergens.*

12:35-13:55 Lunch

Chair: Jolanda van Bilsen & Daniel Lozano-Ojalvo

14:00-14:45 **Keynote lecture: Lotte Neergaard Jacobsen** "EFSA regulation on hydrolysates – consequences for the industry."

14:45 – 15:45 Poster session

15:45-16:15 Coffee Break

Chair: Ben Remington & René Crevel

16:15-16:35 **Antonio Fernandez Dumont**- *New developments for the allergenicity assessment of GM plants.*

16:35-16:55 **Clare Mills** - *In vitro digestibility tests in allergenicity risk assessment of newly expressed proteins in GMO's*

16:55-17:15 **Geert Houben** - *The Threshold of Toxicological Concern; a perspective also applicable to allergenicity?*

17:15-17:35 **Charlotte Madsen** - *Is it possible to define a "Threshold of Concern for Allergic Sensitisation"?*

19:00-22:00 Conference Dinner

Day 3 - Thursday October 12th

08:45-10:00 Working group 1-2-3-4 meeting

Chair: Michelle Epstein & Gregory Bouchaud

10:00-10:45 **Keynote lecture: Jonathan Hourihane** *"If zero risk is impossible, what risk is 'acceptable'? An allergist's view."*

10:45-11:15 Coffee Break

11:15-11:35 **Katrine Graversen** - *Correlation of the allergenicity and tolerogenicity of two cow's milk protein products with their intestinal uptake – a study in Brown Norway (BN) rats.*

11:35-11:55 **Sara Benedé Pérez**- *Unique factors between mice with different clinical manifestations of food allergy determine mast cell response to allergen oral immunotherapy.*

11:55-12:15 **Anne-Sofie Ballegaard** - *Sensitising capacity of five different wheat products through the skin.*

12.15-12.35 **Karine Adel-Patient** - *Food protein induced enterocolitis syndrome (FPIES) induced by cow's milk traduces in local cellular response and in a specific metabolic signature in plasma.*

12:35-13:00 Closing of the conference

13:00-14.00 Lunch

CVs invited speakers

Lecture: “Host-microbe interactions in the gut”
Day 1- Tuesday October 10th 16:20 - 17:05



Maria Rescigno

European Institute of Oncology and University of Milan, Italy

Maria Rescigno graduated in Biology in 1990 at the University of Milan. From 1991 to 1994 she worked at the University of Cambridge, UK, in the Department of Biochemistry, as a visiting scholar. From 1995 to 1999, she worked at the National Research Council of Milan where she received her PhD in Pharmacology and toxicology in 1999. From 1999 to 2001 she worked at the University of Milano-Bicocca where she specialized in Applied Biotechnology. Since 2001 she is the director of the Dendritic cell biology and immunotherapy Unit at the Department of Experimental Oncology at the European Institute of oncology. She was the first to show that dendritic cells in the gut actively participate to bacterial uptake and the existence of a gut vascular barrier that resembles the blood brain barrier. Her major field of interest is mucosal immunology and the development of new cancer immunotherapy strategies. She authored more than 100 publications in high impact journals including Science, Nature Immunol, Immunity, J. Exp. Med., Science TM. She was nominated EMBO young investigator in 2007. Since 2008 she is visiting professor at the University of Oslo. In 2011 Maria Rescigno won the Avon prize as ‘Woman symbol of the city of Milan’ and was elected EMBO member. She has been the recipient of two ERC grants (starting and consolidator). From 2014 she is an associate professor at the University of Milan.

H-index: 50

Lecture: “Have EAT & LEAP left us any clearer as to how we should introduce
allergenic foods to infants in real life?”

Day 2- Wednesday October 11th 10:00 - 10:45



Michael Perkin

St George's Hospital, United Kingdom

Dr Perkin is a Senior Lecturer & Consultant in Paediatric Allergy at St George's Hospital. For eight years he ran the FSA/MRC funded “EAT” (Enquiring About Tolerance) Study the results of which were published in March 2016 in the New England Journal of Medicine. Michael trained at St George's Hospital and then undertook clinical allergy training at St Mary's and Southampton Hospitals. He held a Wellcome Fellowship in Clinical Epidemiology under Professor David Strachan. His PhD was on the relationships between atopy and the farming environment in children. His research interests are the epidemiology of allergic disease, the prevention of food allergy and the environmental contribution to the allergy epidemic.

Lecture: “EFSA regulation on hydrolysates – consequences for the industry”
Day 2- Wednesday 11th 14:00 - 14:45



Lotte Neergaard Jacobsen

Arla Foods Ingredients Group P/S, Denmark

My name is Lotte Neergaard Jacobsen, and I’m a Pediatric Research Scientist at Arla Foods Ingredients P/S (AFI). I have been working in this interesting field for the last 7 years – solely focusing on development and documentation of ingredients for infant nutrition. I have a master degree in Molecular Biology from Aarhus University in 2003, Before joining AFI, I was part of a small biotech company, where transgenic plants produced the B12-binding protein, intrinsic factor for B12-deficient people.

At AFI, I’m responsible for developing a range of scientifically documented ingredients for use in infant formulas, including identification of new proteins, development of new ingredients and documentation of their safety and efficacy.

My areas of interest and expertise are infant immune development and cow’s milk allergy, including the use of hydrolysates for management of allergy. During the last year, especially the cow’s milk allergy area, has been heavily regulated, which is a necessity for ingredients for the very fragile infant segment.

Arla Foods Ingredients P/S is a global leader in milk protein ingredients for products in a range of categories, but with a strategic focus on ingredients for infant nutrition.

Lecture: "If zero risk is impossible, what risk is 'acceptable'? An allergist's view"
Day 3- Thursday October 12th 10:00 - 10:45



Jonathan O'B Hourihane

Paediatrics and Child Health in University College Cork, Ireland

Jonathan Hourihane has been Professor of Paediatrics and Child Health in University College Cork, Ireland since 2005. He graduated from Trinity College Dublin in 1987. He undertook his higher training in Southampton, and London, UK. His primary area of clinical and research interest is in paediatric food allergy and anaphylaxis. He is co-Principal Investigator of the BASELINE, Ireland's only birth cohort study (www.baselinestudy.net.) and the INFANT Research Centre in UCC, and is a founding Board member of the Clemens von Pirquet Foundation, the Irish Food Allergy Network (www.ifan.ie) and is President of the Irish Association of Allergy and Immunology

List of Oral presentations

- O01 A Bayesian network-based approach for discovering.
Jolanda van Bilsen, TNO, The Netherlands
- O02 Characterization of reference proteins: native and modified gliadins for the selection of in vitro model of allergenic risk assessment.
Clélia Villemin, INRA, France
- O03 Non-digestible oligosaccharides can influence IgE-mediated basophil degranulation in whole blood of peanut allergic patients.
Simone Hayen, UMCU, The Netherlands
- O04 Prediction of food protein allergenicity using the GARD assay.
Robin Gradin, SenzaGen AB, Sweden
- O05 Have EAT & LEAP left us any clearer as to how we should introduce allergenic foods to infants in real life?
Michael Perkin, St. George's Hospital, United Kingdom
- O06 Mass spectrometry based proteomic identification of extracellular proteins cleaved by protease activity of actinidin, a major allergen from kiwifruit.
Marija Gavrovic-Jankulovic, Faculty of Chemistry University of Belgrade, Serbia
- O07 Detecting allergens in processed foods: the development of a multi-allergen LC-MS/MS method for baked goods.
Christof van Poucke, Flanders research institute for agriculture, fisheries and food (ILVO), Belgium
- O08 Production, digestibility and allergenicity of hemp (*Cannabis sativa* L.) based ingredients and products.
Pasquale Ferranti, University of Naples, Italy
- O09 Peptide fingerprints in food allergens.
Ivan Dimitrov, Medical University of Sofia, Bulgaria
- O10 EFSA regulation on hydrolysates – consequences for the industry.
Lotte Neergaard Jacobsen, Arla Foods, Denmark
- O11 New developments for the allergenicity assessment of GM plants.
Antonio Fernandez Dumont, EFSA, Spain
- O12 In vitro digestibility tests in allergenicity risk assessment of newly expressed proteins in GMOs.
Clare Mills, University of Manchester, United Kingdom
- O13 The Threshold of Toxicological Concern; a perspective also applicable to allergenicity?
Geert Houben, TNO, The Netherlands
- O14 Is it possible to define a “Threshold of Concern for Allergic Sensitisation”?
Charlotte Madsen, National Food Institute, Technical University of Denmark, Denmark
- O15 *If zero risk is impossible, what risk is ‘acceptable’? An allergist’s view.*
Jonathan Hourihane, Paediatrics and Child Health in University College Cork, Ireland

- O16 Correlation of the allergenicity and tolerogenicity of two cow's milk protein products with their intestinal uptake – a study in Brown Norway (BN) rats.
Katrine Graversen, National Food Institute, Technical University of Denmark, Denmark
- O17 Unique factors between mice with different clinical manifestations of food allergy determine mast cell response to allergen oral immunotherapy.
Sara Benedé Pérez, CISC, Spain
- O18 Sensitising capacity of five different wheat products through the skin
Anne-Sofie Ravn Ballegaard, National Food Institute, Technical University of Denmark, Denmark
- O19 Food protein induced enterocolitis syndrome (FPIES) induced by cow's milk translates in local cellular response and in a specific metabolic signature in plasma
Karine Adel-Patient, INRA, France

List of Poster presentations

- P01 A mouse/human chimeric IgE to assess the allergenicity of Acid –Hydrolyzed Wheat Proteins.
Olivier Tranquet, INRA, France
- P02 Non-digestible oligosaccharides can influence IgE-mediated basophil degranulation in whole blood of peanut allergic patients.
Simone Hayen, UMCU, The Netherlands
- P03 Impact of the Caco2 monolayer crossing on gliadins elicitation capacity.
Colette Larre, INRA, France
- P04 Effect of glycosylation on the allergenicity of invertebrate tropomyosin.
Sibel Karakaya, Ege University, Turkey
- P05 Food Industry and Food Allergens.
Sedef El, Ege University, Turkey
- P06 Impact of food proteins on gut microbiota and immune response in a mouse model of allergy with increased intestine sensitivity..
Dagmara Złotkowska, Institute of Animal Reproduction and Food Research of the Polish
- P07 Immunoreactive properties of α -casein and κ -casein - ex vivo and in vivo studies.
Ewa Fuc, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Poland
- P08 Generation and characterization of two in vitro systems of bone marrow derived mast cells modeling mucosal and connective tissue subsets.
Sara Benedé Pérez, CISC, Spain
- P09 2S Albumins are involved in Cross-reactivity between Pistachio and Cashew Nut in two children.
Cristina Bueno, Universidad complutense de Madrid, Spain
- P10 Effects of interactions with phenolic compounds on the stability of certain allergen proteins
Birgül Hızlar, Ege University, Turkey
- P11 Recombinant tomato allergens: a deeply approach in the diagnostic tool.
Laura Martin-Pedraza, Universidad Complutense de Madrid, Spain
- P12 Aggregation of ovalbumin and allergenicity.
Gregory Bouchaud, INRA, France
- P13 Identification of digestion resistant casein domains after in vitro gastrointestinal digestion followed by the incorporation of the intestinal brush border peptidases.
Lourdes Amigo, Instituto de Investigación en Ciencias de la Alimentación, Spain
- P14 Atibodies to wheat alpha-amylase inhibitor 0.19 in celiac disease.
Daniel Sánchez, Czech Academy of Sciences, Czech Republic

List of Flash presentations

- F01 Generation and characterization of a recombinant nonallergenic chicken tropomyosin as a potential molecular reference for allergenicity assessment studies.
Joana Costa, University of Porto, Portugal
- F02 Comparison of the digestibility and antigenicity of raw and roasted whole peanut allergens.
Luigia di Stasio, University of Naples, Italy
- F03 The effect of Akkermansia muciniphilia on house dust mite induced allergic airway inflammation.
Katrine Graversen, National Food Institute, Technical University of Denmark, Denmark
- F04 Investigation of clinical and immunological reactivity of the major fish allergens parvalbumins.
Tanja Kalic, University of Vienna, Austria
- F05 Food allergy skin sensitization: A comparative study with three different gluten products in Brown Norway rats.
Laure Castan, INRA, France
- F06 Allergen identification in two main Mediterranean fish species using proteomics.
Denise Schrama, University of Algarve, Portugal
- F07 Immunoproteomics characterization of allergenic and non-allergenic tropomyosin orthologs.
Cristian Piras, University of Milan, Italy
- F08 Lupine allergens in food products: a new real-time PCR approach for its detection and quantification.
Caterina Villa, REQUIMTE-LAQV, Faculty of Pharmacy, University of Porto, Portugal
- F09 Detection of soy (Glycine max) allergens in processed model and commercial foods by ELISA techniques: Influence of selected target protein and ELISA format applied.
Isabel Segura Gil, University of Zaragoza, Spain

Abstracts Oral, Poster & Flash presentations

A Bayesian network-based approach for discovering biomarkers for oral immunotherapy of food allergy

Jolanda van Bilsen¹, Lars Verschuren¹, Laura Wagenaar², Marlotte Vonk^{3,4}, Betty van Esch^{3,4}, Léon Knippels^{3,4}, Joost Smit², Raymond Pieters², Tim van den Broek¹

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²Institute of Risk Assessment Sciences, Utrecht University, the Netherlands

³Utrecht Institute of Pharmaceutical Sciences, Utrecht University, the Netherlands

⁴Danone Nutricia Research, Utrecht, the Netherlands

All authors are member of the NUTRALL research consortium

Oral immunotherapy (OIT) is a promising therapeutic approach to treat food allergic patients. Recently, we have shown that the use of non-digestible oligosaccharides improves the efficacy of OIT in cow's milk and peanut allergic mice. However, concerns with regard to safety and long-term efficacy of OIT remain and there is a need to identify novel biomarkers (panels) that predict, monitor and/or evaluate the effects of OIT.

In the present work, we evaluate a computational approach to determine a panel of biomarkers to monitor the efficacy and safety of OIT. For this we exploited data from OIT experiments performed in 3 independent cow's milk allergy experiments and 2 independent peanut allergy experiments in mice.

First, a subset of the data in combination with expert knowledge regarding disease development was used for learning the data structure and their interactions in terms of dynamic Bayesian networks. Next, we formulated and evaluated the performance of the network among OIT-responders versus non-responders and evaluated the efficacy of the use of non-digestible oligosaccharides in OIT. Finally, the relations within the dataset in combination with the Bayesian network were explored to identify and rank candidate biomarkers for the effect of OIT by applying topological analysis methods.

Our analyses show that the approach to combine Bayesian network and topological analyses is promising to identify novel biomarker (panels) of immunotherapy of food allergy.

Characterization of reference proteins: native and modified gliadins for the selection of in vitro model of allergenic risk assessment

Villemain C., Tranquet O., Bouchaud G., Denery-Papini S.

INRA, UR 1268 BIA (Biopolymers, Interactions, Assemblies), F-44316 Nantes, France

Background

Wheat, a highly consumed food worldwide, contains proteins that can cause allergic reactions. In France, the Allergy-vigilance Network reported that wheat was responsible of more than 5% of severe food anaphylaxes in the adult population. Gliadins represent one of the major allergens found in wheat. Wheat proteins may be modified for new functional properties and specific cases of allergy to hydrolyzed wheat proteins have been described. Neo-epitopes induced by an acidic treatment (leading to deamidation) were evidenced for a group of patients with severe symptoms after ingestion of acid-hydrolyzed wheat proteins (Denery-Papini et al., 2012). Previous studies in our team on a mouse model of allergic sensitization also showed that the modification of gliadins by deamidation increased their allergenic potential (Gourbeyre et al., 2012). Similar observations in a rat model showed that acid hydrolysis induces the formation of new epitopes, contrarily to an enzymatic hydrolyzed gluten (Kroghsbo et al., 2014). Thus, native gliadins, acid modified and enzymatically modified gliadins seem to be a relevant panel of reference proteins with various allergenic potential.

The aim of this work was to perform a thorough characterization of this allergen panel in order to use it for in vitro studies.

Method

After selective extraction, native gliadins were enzymatically treated during two hours with alcalase or by heating in acidic conditions. After the treatments, the composition in proteins and polypeptides of the samples were studied thanks to various electrophoreses. Their extent of modification was investigated by ELISA with antibodies directed to native or deamidated epitopes.

Results

The molecular weights (MW) of the native sample varied between 15 and 50 kDa whereas the acid treatment led to aggregation and a limited hydrolysis with MW in the range of 10-75 kDa and our enzymatic treatment led to strong proteolysis (MW < 10 kDa). The estimated degree of deamidation increased with the deamidation time and was in inverse proportion to the native residual sequences.

Conclusion

This panel of samples displayed very different biochemical characteristics which may be linked to their weak or high allergen potential. Other determinations still need to be carried out such as endotoxin quantification and analyses by chromatography of the mass distribution and presence of small aggregates.

Combined exposure of intestinal epithelial cells to dietary oligosaccharide mixture scFOS/lcFOS and CpG DNA effectively enhances the Th1 and regulatory IL-10 response in a peanut-specific co-culture model

S.M. Hayen^{1,2}, S.A. Overbeek³, A.C.Knulst¹, J.Garssen^{3,4}, H.G. Otten², L.E.M Willemsen³

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²Laboratory of Translational Immunology, University Medical Center Utrecht, Utrecht, The Netherlands

³Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht, The Netherlands

⁴Nutricia Research, Immunology, Utrecht, The Netherlands

Background: The intestinal mucosa plays a key role in the development of food allergies. In a transwell co-culture model, exposure of IECs to a oligosaccharide mixture of scGOS/lcFOS in combination with synthetic TLR-9 ligand CpG ODN, modulated the cytokine response of activated PBMCs of healthy donors, driving away from the allergic phenotype. Since scGOS is derived from lactose, it may pose risks in cow's milk allergic patients. Therefore, this study compared the efficacy of scGOS/lcFOS with scFOS/lcFOS and evaluated these effects in an allergen-specific co-culture model, with PBMCs of peanut-allergic donors.

Method: IECs grown on transwell filters were apically exposed CpG ODN either or not combined with scGOS/lcFOS or scFOS/lcFOS. These IECs were co-cultured with PBMCs from peanut-allergic donors, either stimulated aspecifically (α-CD3/28, 24 hours) or with crude peanut extract (CPE, 6 days). Readout parameters were cytokine production and T cell polarization.

Results: Apical exposure of IECs to CpG ODN enhanced basolateral IFN-γ and IL-10 production by anti-CD3/28 activated PBMCs (p<0.01), while IL-13 and TNF-α production decreased. Both oligosaccharide mixtures further enhanced IFN-γ and IL-10 production (p<0.05). CpG exposure in the allergen-specific transwells also increased IFN-γ and IL-10 production by PBMCs, which was only further enhanced by scFOS/lcFOS (p<0.05). This coincided with increased Th1 frequency.

Conclusion: Epithelial exposure to both scGOS/lcFOS and scFOS/lcFOS enhances the CpG DNA induced Th1 and regulatory IL-10 response in an anti-CD3/CD28 co-culture model, whereas only scFOS/lcFOS was effective in a peanut-specific co-culture model. Thus scFOS/lcFOS may be an interesting candidate in dietary adjunct therapy for allergen-specific immunotherapy.

Prediction of food protein allergenicity using the GARD assay

Robin Gradin¹, Maria Agemark¹, Angelica Johansson¹, Olivia Larne¹, Kathrin Zeller², Malin Lindstedt², Erwin Roggen^{1,3}, PATC⁴, Henrik Johansson¹

¹SenzaGen, Sweden

²Lund University, Sweden

³3Rs Management and Consulting, Denmark

⁴Protein Allergy Technical Committee (PATC) of the ILSI Health and Environmental Science Institute

Background

Genomic Allergen Rapid Detection (GARD) was originally developed as an in vitro assay for the assessment of skin sensitizers. GARD uses the transcriptional response of a myeloid cell line to identify sensitizing substances. Further development of the assay has aimed to broaden the applicability domain of its hazard classifications. A novel biomarker signature, the protein allergen prediction signature (PAPS), was identified in a previous study which separated protein allergens from non-allergens. Here we present results when challenging the prediction model with two food proteins, tropomyosin originating from shrimp and tropomyosin originating from pork.

Method

Total RNA was isolated from MUTZ-3 after 24 h of exposure to tropomyosin originating from pork and shrimp. An LPS control was also included to verify that background levels of endotoxins did not produce false positive classifications. Cellular stimulations were performed in triplicates. Expression levels were quantified with Affymetrix microarrays and normalized with SCAN. The genes in the PAPS were extracted and an SVM, defined in the previous study, was used to classify each protein as allergen or non-allergen.

Results

The allergenic tropomyosin purified from shrimp was correctly classified as a protein allergen and the non-allergenic tropomyosin originating from pork was correctly classified as a non-allergen. The LPS control was classified as a non-allergen.

Conclusion

The recently identified GARD PAPS was challenged by classifying two previously unseen proteins, one allergen and one non-allergen. The resulting predictions correctly classified both proteins in their respective category. These results are promising for the development of a functional in vitro assay for the assessment of protein allergens. It also further demonstrates the versatility of the GARD platform to target different endpoints using specific transcriptional signatures..

Have EAT & LEAP left us any clearer as to how we should introduce allergenic foods to infants in real life?

M. Perkin

St George's Hospital, United Kingdom

The World Health Organization (WHO) Global Strategy for Infant and Young Child Feeding, endorsed by the UK Government, recommends exclusive breastfeeding for the first six months with nutritious complementary foods introduced thereafter and continued breastfeeding up to the age of two years or beyond. The UK Government adopts a more pragmatic target of around six months exclusive breastfeeding. It also states that if a mother decides to introduce complementary foods before six months, there are some foods that should be avoided as they may cause allergies including: "wheat-based foods...eggs, fish, shellfish, nuts (and) seeds." When allergenic foods are introduced it recommends that this be done one food at a time to detect reactions.

Between 1998 and 2009 the United Kingdom government had a more restrictive policy, recommending avoidance of peanut consumption in high-risk families during pregnancy, lactation and to the child until three years of age. The American Academy of Pediatrics from 2000 to 2008 also recommended high-risk infants avoid solids until six months of age, dairy products until one year of age, hen's egg to two years and peanuts, tree nuts and fish to three years of age. However the evidence basis for both the instigation and the revocation of these guidelines was limited.

However, there it was the emergence of observational evidence that the introduction of cow's milk, egg or peanut during infancy may prevent the development of food allergies that has led to a number of randomized trials testing the hypothesis that the early introduction of allergenic food can prevent food allergy from developing.

Two of the most important have been the LEAP and EAT studies, based in the UK. Other studies have been published from trials in Australia, Japan and Germany. This talk will review the evidence from these trials and discuss the implications of the results.

With countries now changing their infant feeding guidelines, with widely different recommendations being made, this talk will explore whether we are indeed any clearer as to how and when we should be introducing allergenic foods into infants' diets.

Mass Spectrometry based proteomic identification of extracellular proteins cleaved by protease activity of actinidin, a major allergen from kiwifruit

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The molecular mechanisms of primary sensitisation to allergens are not completely understood at molecular level. The gastrointestinal tract, as a largest organ of immune system, is the first in contact with food allergens. The role of protease activity of allergens in this paramount process in allergy was recently envisaged by several groups and degradation of tight junction protein occludin was documented [1-3]. Proteolytic shedding of extracellular domains of transmembrane proteins by cysteine protease was documented [4]. We further investigate the molecular mechanism of cysteine protease actinidin (Act d 1) action on the intestinal epithelium using Caco-2 cell monolayer. Mass spectrometry-based proteomic shotgun approach was applied on nanoHPLC(C18)-nanoESI-LTQ-Orbitrap Velos MS system. Repertoire of surface and transmembrane proteins are identified as potential physiological substrates of actinidin. Moreover, some of them that are not known as surface proteins are identified in “surfome”. Potential role of identified physiological substrates of actinidin in mechanism of sensitisation was discussed.

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Detecting allergens in processed foods: the development of a multi-allergen LC-MS/MS method for baked goods.

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Background

LC-MS/MS is gaining popularity for the detection of allergens in food as it can overcome several of the problems encountered with ELISA and PCR (comparability of results, cross reactions, ...), moreover LC-MS/MS had the advantage of multiplexing. This method is however also susceptible to the impact of food processing on the detectability of allergens. It is therefore important to make sure that the targeted allergens have undergone this processing when developing an LC-MS/MS method.

Method

Two sorts of doughs were prepared: one containing each of the allergens milk, egg, soy, peanut, hazelnut, pecan and mustard at a concentration of 10000 mg allergen / kg dough, and a second dough free from the above mentioned allergens. Both doughs were baked and the obtained cookies were grinded. A serial dilution of the allergen containing cookie was made with the allergen free cookie to obtain concentrations ranging between 0 – 5000 mg allergen / kg cookie.

For LC-MS/MS analysis 2.5 gram cookie was extracted with 10 mL extraction buffer. Following reduction and alkylation, extracts were desalted using gel filtration and trypsin digested to obtain mass spectrometric compatible peptides. The tryptic digest was further purified and concentrated using C18 SPE. Selection of marker peptides was made *in silico* based on data available on the Allergen peptide browser website¹ and skyline fragmentation².

Results

Preliminary tests indicated that desalting using gel filtration after reduction/alkylation gave far better sensitivity than reducing the salt concentration by dilution prior to trypsin digestion. A drawback to the gel filtration is the limited amount of solvent that can be used on each column. By optimizing the volume of the extraction solvent however a higher protein concentration in the extract was obtained and as a result a larger amount of proteins could be digested.

Not all selected marker peptides were detectable in the baked cookies and further refinement was done based on the obtained data, selecting the proteotypic peptides which allowed the lowest detection limits for each allergen.

Conclusion

The developed multi-allergen LC-MS/MS method is able to detect the targeted allergens at levels ranging between 0.85 and 3.15 mg total protein / kg cookie. These levels are, with the exception of egg and mustard, sufficiently low to allow detection at VITAL levels for a portion size of 40 g cookies³.

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Production, digestibility and allergenicity of hemp (*Cannabis sativa* L.) based ingredients and products

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Background. Hemp (*Cannabis sativa* L.) is cultivated for industrial use and harvested for fibers, seeds, oil and meal, and raised much interest as a sustainable cultivation. For these reasons, hemp seeds have started to be used in a variety of food products. Hemp-based food products are considered less allergenic than those from other edible seeds, although this statement has never been experimentally confirmed.

Methods. A high purity grade HPI was obtained by a fast and cheap process, enriched at nearly 80% of the protein edestin. In vitro digestibility was determined using a static model of gastrointestinal digestion (GID) which included a final step with purified brush border membrane enzyme preparations.

Results. The HPI showed a high degree of digestibility confirmed also by Western blotting, whereas amino acid analysis showed well-balanced levels of essential amino acids. In order to evaluate survival of bioactive and/or allergenic sequences, the product of GID were characterized by peptidomics. Only a limited number of sequences survived gastrointestinal digestion. Among them, fragments from 12 seed proteins. They were precursors of sequences with antimicrobial, antioxidant or neuroactive peptides, which can justify the bioactivity of HPI hydrolysates, reported by previous studies. More importantly, the major allergen form Hemp, thaumatin-like protein/LTP, was entirely eliminated by the HPI production process, neither fragments of the proteins were present after GID.

Conclusions. Data supported the use of hemp and HPI as ingredient for hypoallergenic foods. On these bases, we developed a panel of novel, entirely hemp-based products such as desserts and drinks with excellent sensory properties. Fiber- and protein- rich wastes from this process have been also recovered to produce baked goods such as biscuits and bars. Scaling-up of the process to industrial scale may result in the development of novel hypoallergenic products which also meet sustainability requirements.

Peptide fingerprints in food allergens

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Background

Food allergens are degraded in gastro-intestinal tract (GIT) by different proteases. As a result, peptides of different length are generated, absorb, enter the antigen-processing cells (APCs) and bind to HLA class II proteins. The peptide-HLA complex is presented on the cell surface, where is recognized by CD4+ T cells and initiates an immune response.

In the present study, we develop an algorithm mimicking the allergen digestion in GIT followed by HLA class II binding of peptide fragments. The high affinity peptides are compiled into a database and used to derive binary fingerprints based on the physicochemical properties of the amino acid residues.

Methods

In silico allergen digestion was performed by PeptideCutter server using the enzyme pepsin. The binding affinities to 12 HLA-DRB1, 5 HLA-DQ and 7 HLA-DP proteins were evaluated by EpiTOP server. The amino acid physicochemical properties were described by five *E*-descriptors. Tanimoto similarity index was used to identify common fingerprints.

Results

A set of 2491 known food allergens from 336 species underwent *in silico* digestion by pepsin. The digested fragments were presented as overlapping nonamers and their affinities to 24 HLA class II proteins were evaluated by EpiTOP server. The high affinity peptides (IC₅₀ < 500 nM) for each allele were encoded by amino acid five *E*-descriptors and transformed into binary fingerprints. The fingerprints were compared by Tanimoto index and common fingerprints for each HLA were derived.

Conclusion

The *in silico* derived common fingerprints in food allergens are able to predict sensitization to novel allergens and cross-reactivity between species.

O10

EFSA regulation on hydrolysates – consequences for the industry

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In 2016 the EU regulation on infant formulas and follow-on formulas was updated, and the requirements for formulas manufactured from hydrolysates were sharpened. Because of the solid documentation package needed, the new Directive will not be effective until February 2021.

Also in 2016, the European Commission requested European Food Safety Authority (EFSA) to provide a “scientific and technical guidance for preparation and presentation of Infant formula/follow-on formula containing protein hydrolysates”. It described how to prepare the documentation package (safety/suitability and efficacy data) needed to market an infant formula containing hydrolysed proteins claiming allergy reduction.

This talk will give an overview of the new EU regulation and the EFSA guidance, and highlight some of the consequences it may have for the infant formula industry

New developments for the allergenicity assessment of GM plants

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The European Food Safety Authority (EFSA) has recently published a document providing supplementary guidance¹ for the allergenicity assessment of genetically modified (GM) plants addressing non-IgE-mediated adverse immune reactions to food and endogenous allergenicity. In particular, a risk assessment strategy for the assessment of (novel) proteins with regard to celiac disease, a non-IgE-mediated adverse reaction to foods, is provided for the first time. For this assessment, a stepwise approach is proposed where knowledge of the (novel) protein under assessment is used as starting point to calibrate the subsequent risk assessment strategy which is undertaken on a case-by-case basis. Depending on the outcome of the information retrieved, searches for sequence identity can be used. If concerns from this analysis are identified, in a second step, HLA-DQ peptide modelling can be applied. In a third step, *in vitro* tests such as HLA-DQ-peptide binding assays and/or testing with T-cell clones derived from patients with celiac disease can be carried out to determine the safety profile of the (novel) protein. In relation to endogenous allergenicity, the measurement of relevant endogenous allergens is mandatory with the EU Implementing Regulation 503/2013. The new EFSA document provides detailed guidance on how to address this requirement. Specifically, the guidance elaborates on the following topics: i) relevant crops for analysis, considering their regulatory status and/or their public health importance as the starting point in the assessment; ii) relevant allergens for quantification, being international recognition and/or clinical relevance the main aspects to consider for the selection of allergens; iii) methodology for quantification, where ELISA and mass spectrometry are appropriate methods for the quantification of individual allergens; and iv) data interpretation and risk assessment, providing additional considerations taking into account the number and magnitude of the changes identified as well as the clinical relevance of the specific allergen involved. In this guidance document, EFSA also describes a refined *in vitro* protein digestion test for the safety assessment that will undergo an interim phase before any additional recommendations in the form of guidance for applicants can be provided. This is considered a major step forward with a great potential for the safety assessment of (novel) proteins and is the subject of an additional abstract in this conference.

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In vitro digestibility tests in allergenicity risk assessment of newly expressed proteins in GMOs

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Since the development and widespread adoption of the pepsin resistance test in the 1990's *in vitro* digestibility tests have been used to provide data for the integrated allergenicity risk assessment of newly expressed proteins in GMO's. However, it is acknowledged that the test has limitations, especially when used as a stand-alone analysis, partly because of the very low pH employed in the assay and the gross excess of pepsin to protein substrate used. Furthermore the correlation with protein allergenicity has been questioned since studies have demonstrated that food allergens were not always inherently more stable to pepsin digestion than non-allergenic proteins in the test. However, since there is evidence that gastrointestinal digestion can affect the immunogenicity of dietary proteins it has been suggested other *in vitro* digestibility methods designed to more closely simulate the conditions of the human digestion process may also have value for the risk assessment. Based on an assessment of the current state-of-the-art a refined *in vitro* digestion test has been proposed which extends the conditions currently used in the classical pepsin resistance test in order to better reflect the range of conditions found *in vivo*. As part of this activity an assessment was made of the data available on the composition of human digestive fluids which could inform identification of relevant test conditions. Since these are generally obtained from analysis of aspirates taken through invasive procedures data from healthy individuals are sparse, especially with regards compositional analysis. It was found that good quality data were available on intragastric pH ranges in adults and children in the fasted and fed-states. Furthermore, whilst gastric and intestinal pH values can be derived from direct measurements, the levels of digestive enzymes have been reported using a variety of different and non-standardised assays, and activities reported using different units or as flow rates. This makes interpretation of such data challenging and the identification of appropriate pH conditions and well-defined levels and activities of digestive enzymes to be included in *in vitro* test systems difficult. In addition to defining test conditions, methods are needed to characterise the length, persistence and abundance of peptides derived from *in vitro* gastrointestinal digestion of newly expressed proteins. The kinetic parameter of "half-life" could provide a means of defining "transient" and "persistent" peptides generated by a given set of digestion conditions and has been used in assessments of the allergenic risk of novel proteins, such as the ice structuring protein. However, further evidence to support the application of such a parameter needs to be obtained and to allow appropriate test conditions and end-points of digestion to be assessed before any additional recommendations regarding *in vitro* digestion tests can be made.

O13

The Threshold of Toxicological Concern; a perspective also applicable to allergenicity?

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Already ages ago, it has been recognised that any substance can cause toxic effects, if exposure is high enough, and that low doses make substances not to be poisonous. Knowledge regarding dose-effect relationships in toxicology has allowed this recognition to be developed into the concept of the Threshold of Toxicological Concern (TTC), i.e. the elaboration of thresholds of exposure below which no toxicological health risks are expected. During the past 1,5 decade, this concept has been refined through the elaboration of separate thresholds for subgroups of chemicals. The TTC concept and elaborated thresholds will be presented and the question will be addressed whether such concepts might also be developed for food allergenicity.

Is it possible to define a “Threshold of Concern for Allergic Sensitisation”?

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One of the features generally NOT taken into account in risk assessment, when assessing the sensitising capacity of food proteins, is dose. WG4 has therefore decided to look into this.

We know, that the normal condition is to develop oral tolerance to food proteins, and that not all individuals are at equal risk of getting sensitised, but we know little about the conditions necessary for sensitisation via the oral route, including dose.

From *contact sensitisation to chemicals* we know that there, for a specific chemical, is a dose/cm² that is too low to sensitise, because the impact on the immune system is too low to initiate a response. We also know, that the dose necessary to sensitise is higher, than the dose eliciting a response in an already sensitised subject.

Common for *protein allergens in foods*, where sensitisation via the GI tract occurs, is that they are abundant in the food, and are a part of the normal diet. We therefore *hypothesise*, that dose and frequency of exposure are important parameters for sensitisation i.e. that without a sufficient dose and perhaps a sufficient frequency of exposure, sensitisation will not occur.

To *test the hypothesis* we need data for specific foods on prevalence of sensitisation, route of sensitisation, sensitising allergen, abundance of the allergen in the food, and the amount and frequency of consumption of the food.

Using hazelnuts, milk and egg as examples available data on sensitisation, abundance of allergens and consumption will be presented. The possibility of testing the hypothesis will be discussed.

If zero risk is impossible, what risk is ‘acceptable’? An allergist’s view

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Allergy risk assessment and allergen risk management have been to the fore in the modern era of blame displacement by consumers and reciprocal risk transference by corporations to consumers. In the last 20 years there have been rounds of joint development of risk management strategies involving all common stakeholders in this field.

Manufacturers cannot realistically guarantee bulletproof zero allergen contamination and the initially absolute zero risk acceptance by consumers has gradually moved to a more dynamically manageable understanding of risk minimisation, which must involve transparency and information from providers and personal responsibility from allergic consumers.

Clinical allergists have engaged in all of these developments and have developed low dose food challenge methodology to inform this area with data based on a wide range of allergy phenotypes and range-finding studies including low dose graded challenges and recently single dose challenge studies.

Correlation of the allergenicity and tolerogenicity of two cow's milk protein products with their intestinal uptake – a study in Brown Norway (BN) rats

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Background: It remains largely unknown which features of food proteins that render them allergenic versus tolerogenic. However, it has been suggested that protein-chemical properties, in particular aggregation status, affect protein uptake in the intestine, and that uptake route may impact on the risk of sensitisation. The aim of this study was to investigate the interplay between protein-chemical features, the allergenic versus tolerogenic properties and the intestinal uptake of two whey products.

Method: The allergenic versus tolerogenic capacity of a heat-treated whey product, containing partly denatured and aggregated proteins, was compared to the same untreated product in BN rat models of: 1) i.p. and 2) oral sensitisation, 3) oral primary prevention and 4) oral desensitisation. Elicitation was measured by *in vivo* tests and antibody responses by ELISAs. The intestinal uptake was compared by quantifying BLG levels in small intestinal tissues at different time points after oral dosing of the two products in naïve BN rats. The findings were supported by *in vitro* uptake experiments in cell cultures.

Results: Unmodified and heat-treated whey had similar tolerance inducing and de-sensitising capacity, but the sensitising and eliciting capacity of the heat-treated product was significantly reduced compared to the untreated product, despite the immunogenicity of the two products were similar. Collectively the *in vivo* and *in vitro* uptake experiments suggested that uptake kinetics and the major intestinal uptake route differed between the two products.

Conclusion: Heat-treatment, which induced partly protein aggregation, changed the immunological properties of a whey protein product. This might in part be explained by the different intestinal uptake of the unmodified and heat-treated products. The heat-treated product had a reduced allergenicity combined with a high tolerogenicity, which highlights this product's promising potential for induction of cow's milk tolerance.

Unique factors between mice with different clinical manifestations of food allergy determine mast cell response to allergen oral immunotherapy

Sara Benedé & M. Cecilia Berin

BACKGROUND: Mast cells are key effector cells of allergic reactions, and are classified into mucosal (MMCs) and connective tissue (CTMCs) mast cells. The function of these different mast cell subsets in food allergy remains unknown.

METHODS: We evaluated MMCs and CTMCs activation by release of the protease MMCP-1 and -7 respectively, using two different mouse models of food allergy with gastrointestinal or anaphylaxis symptoms after oral ovalbumin challenge. Anaphylaxis severity was measured by drop in body temperature, gastrointestinal symptoms (diarrhea) were marked as present or absent. Mice with symptoms underwent an oral desensitization protocol by feeding antigen for 2 weeks.

RESULTS: Tissue restriction of MMCP1 and MMCP7 in gut and skin, respectively, was confirmed by qPCR and immunohistochemistry. Using sensitized mice, we took advantage of responders and non-responders in the two models of food allergy to study the association of MMCs and CTMCs activation with symptoms. We observed that anaphylaxis was significantly associated with release of histamine and CTMCs, but not MMCs, activation. In contrast, diarrhea was associated with both, MMCs and CTMCs activation. Symptoms in response to oral challenge, and challenge-induced MMCP-1 and MMCP-7 were both significantly suppressed by oral immunotherapy in mice from the anaphylaxis model, indicating that both MMCs and CTMCs can be desensitized to prevent anaphylaxis *in vivo*. In contrast, administration of oral immunotherapy to mice with gastrointestinal symptoms exacerbated both CTMCs and MMCs activation in response to oral challenge.

CONCLUSION: In summary, the data presented in this paper demonstrate the clinical importance of MCs heterogeneity in the context of food allergy. These findings show that although MMCs and CTMCs have the potential to be desensitized, there are unique factors between different manifestations of food allergy that determine their response to allergen immunotherapy.

Sensitising capacity of five different wheat products through the skin

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Background: Allergic sensitisation to foods may occur in infancy without prior oral exposure to the offending food. This has led to the assumption that food allergy sensitisation may occur through alternative routes, such as the skin. Recently, concerns have been raised regarding the safety of use of cosmetics and personal care products containing hydrolysed wheat proteins, since these products have been shown to induce allergy towards acid hydrolysed wheat through the skin.

Methods: The aim of the study was to compare the sensitising capacity of five different wheat products; an unmodified gluten product, an enzyme hydrolysed gluten product, and three acid hydrolysed gluten products.

Their sensitising capacity and the dose response-relationship was evaluated in a Brown Norway rat skin sensitisation model. Rats were bred and raised on a gluten free diet. Products were applied on slightly damaged skin for one hour per day for three consecutive days. This was repeated for five weeks and rats were subsequently given two oral post-immunisations.

Results: All five gluten products were able to induce a specific antibody response and sensitise through the skin after application on the slightly damaged skin without any use of adjuvant. All products showed a clear dose response-relationship where the highest dose gave the highest response. On the basis of inhibitory ELISA cross-reactivity between the products were examined and differences could be observed between the products. Acid hydrolysed gluten behaved differently than the enzyme hydrolysed gluten indicating that new epitopes had been developed. Furthermore, it seemed that the amount of new epitopes that had been developed was dependent on the degree of acid hydrolysis.

Conclusion: This study showed that all five gluten products were able to sensitise through slightly damaged skin. The response was dependent on the dose applied on the skin together with the degree of modification of the different wheat products.

Food protein induced enterocolitis syndrome (FPIES) induced by cow's milk traduces in local cellular response and in a specific metabolic signature in plasma

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Background: Protein-induced enterocolitis syndrome (FPIES) is a non-IgE mediated food allergy of increasing prevalence for which immune mechanisms are not elucidated and no biomarkers are available.

Objectives: We aimed 1) to further characterize immune response and 2) to carry complementary and innovative approaches using metabolomics to go deeper in pathogenesis and to identify specific biomarkers of cow's milk induced-FPIES (CM-FPIES)

Methods: Children with active CM-FPIES (n=9) or active IgE-mediated CM allergy (IgE-CMA, n=6; also avoiding CM consumption) were recruited in Hospital Necker day care Unit when visiting to perform an oral food challenge (OFC). Blood samples were collected before the OFC. Plasma from age-matched patients tolerant to CM but presenting an IgE-mediated peanut allergy (n=6) were used as controls. The total and CM-allergens specific IgE, IgG1-4, IgA, IgM and IgD were assessed in all plasma, as the same as IgE and IgG4 specific to digestats obtained after in vitro gastric and gastro-duodenal digestion of CM. Specific ex-vivo stimulation of PBMC from CM-FPIES and IgE-CMA children were performed and cytokine secretion and cellular proliferation induced analyzed. Additionally, T cells and innate lymphoid cells (ILC) were analyzed in intestinal biopsies obtained from children with active (n=2) vs non active (n=2) FPIES. Finally, individual metabolic profiles were obtained using two complementary LC-MS methods analysis on plasma from CM-FPIES patients and compared to that obtained from patients suffering from active or resolved IgE-CMA (negative OFC, n=3).

Results: Systemic antigen-specific T-cells and humoral responses were evidenced in IgE-CMA patients whereas they were weak or even absent in CM-FPIES patients, an absence of response that cannot then be restricted to a lower exposure to cow's milk. Preliminary data evidenced activated Th1, Th2 and Th17 cells and activated innate lymphoid cells showing a mixed ILC1/2 phenotype within the intestinal mucosa of active FPIES. Moreover, discriminant statistical analysis of metabolic profiles clearly separated CM-FPIES versus IgE-CMA patients. Further univariate analysis allowed the identification of discriminant metabolites, demonstrating notably dysregulation of fatty acids metabolism in CM-FPIES children.

Conclusions: Children with CM-FPIES have a weak or absent systemic specific immune responses. Our preliminary data suggest that new studies analysing innate and adaptive cells in the mucosa may help to delineate the pathophysiology of FPIES. Metabolomics could further help to identify biomarkers for FPIES, by highlighting altered metabolic pathways.

P01

A mouse/human chimeric IgE to assess the allergenicity of Acid –Hydrolyzed Wheat Proteins

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Acid-hydrolyzed wheat proteins (a-HWP) were used as functionalized ingredients in food and cosmetics. They have been shown to elicit severe and new allergy in Europe and Japan. Acid-hydrolysis of wheat proteins induces random deamidation of their glutamine-rich repeated sequences. Some of these deamidated sequences have been identified as neo-epitopes responsible for this particular allergy to wheat. Depending on acid-hydrolysis condition, deamidation rates vary among acid-HWP samples. This work investigated the capacity of a chimeric IgE to assess the allergenicity of acid-HWP in a basophil assay.

Humanized basophils (RBL SX38) were sensitized with sera from HWP-allergic patients and stimulated with 5 industrial HWP samples and 3 gliadins samples with different level of deamidation. Concentration of HWP or deamidated gliadins which induced 50% of the maximum degranulation (EC50) was determined for each sample. A chimeric mouse/human IgE directed to the main epitope involved in HWP allergy was produced (chIgE-DG1). Reactivity of human sera and chIgE-DG1 was compared in ELISA and in the basophil assay.

All deamidated gliadins and HWP samples induced degranulation of basophils sensitized with patients' sera with different range of response (EC50 from 2 to 333 ng/mL). The chIgE-DG1 and human sera displayed similar reactivity in ELISA. The chIgE-DG1 allowed cell degranulation after stimulation with deamidated gliadins or with industrial HWP samples (EC50 from 13 to 1129 ng/mL), demonstrating its functionality. The samples can be ranked according to their EC50 which reflect their eliciting potential. ChIgE-DG1 and human sera classified the samples in the same order.

The eliciting potential of a-HWP samples spread over 2 orders of magnitude. The chIgE-DG1 was equivalent to patient sera in its ability to classify and rank industrial deamidated glutens in a basophil activation assay and could be a relevant tool in the allergenicity evaluation of functionalized glutens

Non-digestible oligosaccharides can influence IgE-mediated basophil degranulation in whole blood of peanut allergic patients

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Background: Previous research indicated an important role for dietary non-digestible oligosaccharides (NDOs) in decreasing the incidence of atopic dermatitis (AD) in children at risk of allergy. These NDOs can promote colonization of beneficial bacteria in the gut. Enhanced serum galectin-9 levels were found in children with AD receiving a diet of scGOS/lcFOS with *Bifidobacterium breve* M-16V. Galectin-9 has IgE-binding capacities and can hereby suppress degranulation of mast cells and basophils. NDOs may also affect immune cells directly, since they were found in plasma and urine. This study investigated whether NDOs or galectin-9 can have direct effects on basophil degranulation in peanut-allergic patients.

Methods: Whole heparinized blood samples from 12 peanut-allergic patients were incubated with scGOS/lcFOS, scFOS/lcFOS, or galectin-9 (1 or 5 µg/ml) at 37°C in the presence of IL-3 (0.75ng/ml). After 24 hours, a basophil activation test (BAT) was performed. Basophils were stimulated with increasing concentrations of peanut extract or human anti-IgE. Degranulating basophils were calculated as percentage CD63+ cells.

Results: The peanut or anti-IgE concentration that induced maximal degranulation in the control sample of each patient was set to 100%. Pre-treatment of whole blood with scGOS/lcFOS resulted in an average decrease in degranulation of approximately 8%, while a significant reduction of 19% was observed after pre-treatment with scFOS/lcFOS ($p < 0.05$). Pre-treatment with 1 µg/ml galectin-9 decreased basophil degranulation with 12%, whereas 5 µg/ml galectin-9 caused a significant decrease of 25% ($p < 0.05$). Basophils were not less sensitive towards CPE or anti-IgE, since the EC50 was not altered.

Conclusion: The prebiotic mixture scFOS/lcFOS and galectin-9 can contribute to decreased degranulation of basophils in a IgE-mediated BAT assay using whole blood. Further analysis is warranted to define the exact working mechanism of these oligosaccharides.

Impact of the Caco2 monolayer crossing on gliadins elicitation capacity

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Context: Wheat is one of the most important crops in the world in terms of human nutrition. As regard to health aspects, some individuals exhibit wheat-related disorder such as food allergy to wheat (FAW). In this disorder, gluten is involved and in particular the gliadins, that are the main proteins responsible for FAW. Food processing, as well as digestibility and intestinal transport, are key factors to consider since they may affect the allergenic potential of food allergens.

Objective: Because wheat is always consumed after heat process, we investigate the effect of heating and digestion on total gliadins for their capacity to maintain their allergenic potential. In our study, several steps of the “antigen transformation” were taken into account, from heating treatment to gastric digestion before considering the passage of the intestinal barrier.

Material and methods: The processed proteins (heated and heated and digested by pepsin) were characterized for their size by laser light scattering. The chromatographic profiles of the soluble fractions were obtained by RP-HPLC chromatography. The IgE-binding capacity of native and processed proteins was checked by Dot Blot with sera from wheat allergic patients. Furthermore the capacity of these samples to cross the intestinal barrier and to induce the mast cell degranulation was investigated by combining two in vitro cellular models, Caco-2 and RBL-SX38.

Results: The heat treatment of gliadins induced the production of large aggregates that were hardly recognized by patients IgE. However after limited pepsin hydrolysis they recovered partial IgE-binding by unmasking epitopes in dotblot but were not able to trigger RBL cells. A low percentage of native proteins and processed forms crossed the Caco2 cells. After crossing processed proteins recovered in a certain extend their degranulation capacity. Among the set of fractions tested, total native gliadins remained the best allergenic form.

Effect of glycosylation on the allergenicity of invertebrate tropomyosin

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Food allergy is one of the major health concerns worldwide that has been increasing at an alarming rate in recent years. Foods undergo various processing steps before consumption that could affect conformation of food proteins, their digestion and thereby allergenicity. Different processing methods alter the structure of food proteins in different ways and possible structural modifications include unfolding, aggregation, cross-linking between the ingredients and chemical modifications such as oxidation and glycosylation. Conformational changes occurred during food processing can directly influence the allergenicity by disrupting conformational or linear epitopes.

Conformational epitopes can be exposed or hidden by unfolding or aggregation of proteins, whereas sequential epitopes can be affected by acidic or enzymatic hydrolysis and extreme Maillard reactions. Tropomyosin (TM), which is the major allergen of invertebrates, has been noted as one of leading causes of allergic reactions. There are many studies investigating the effects of food processing on TM allergenicity. Among these heat processing, high-pressure treatments and ultrasound application are the most applied methods. There are a few studies regarding effect of glycation on TM allergenicity. Glycation is one of the reaction occurred during food processing and causes the production of diverse Maillard reaction products in the different food matrixes.

One of the studies on TM allergenicity reported that IgE reactivity and the mediator release capacity of scallop TM were enhanced at the early stage of the Maillard reaction, although the effect depended on the type of reducing sugar. In contrast, IgE reactivity of squid TM decreased due to structural changes in the allergen by heat treatment rather than blocking of lysine residues. Another study reported that glycation caused a decrease in the antigenicity and potential allergenicity of shrimp TM and this decrease is well correlated with the conformational changes in its structure. It was concluded that key factors possibly affecting the influence of glycation on IgE reactivity were i) alteration in the protein structure of allergen, i.e., unfolding by heating, and subsequent refolding, oligomerization and aggregation by cooling, ii) changes in the electric charge, and hydrophobicity, and/or structures of proteins thereby changes in affinity and/or accessibility of allergens for specific IgE antibodies, iii) IgE epitopes may be masked due to high level of glycation and this masking may reduce the accessibility for specific IgE antibodies, and subsequently abrogate potential allergenicity of food allergens.

In conclusion, glycation can be useful to reduce allergenicity of TM but further studies investigating different protein:sugar ratio, temperatures and times are needed.

Food Industry and Food Allergens

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Nowadays food industry offers diverse food products to broad range of population including infants, adolescents, adults and elderly. People meet the processed foods in the very early childhood period using infant formulas. In addition, new generations are less aware of traditional basic cooking techniques, or take no time to cook.

Furthermore, a wide variety of precooked and processed foods are now sold. Unfortunately due to the growing complexity of food formulations and food processing, foods may be unintentionally contaminated with allergen-containing ingredients. The only way to verify whether foods are free of traces of allergens is by using reliable analytical methods, which would allow accurate detection in the raw materials and end products. It was long known that detection of food allergens is hampered by the presence of matrix components. The food matrix can mask food allergen in different ways. First of all the matrix can bind the analytical target (proteins/allergens) therefore hampering their extraction. Matrix components are prone to form covalent, ionic, and hydrogen bonds or hydrophobic interactions with target proteins. This was proven in the study where cow's milk proteins, caseins, bound to the matrix making their extraction inefficient especially in low concentration ranges. This indicated that detection of especially trace amounts of allergens would be severely affected regardless of the type of analytical method used.

Besides, food processing can cause changes in the food matrix and/or result in increased or decreased allergenicity of proteins either by the formation of new IgE binding epitopes or by the destruction of existing ones. Such modifications include unfolding, aggregation, fragmentation, hydrolysis, covalent modification, or total degradation, ultimately altering the protein epitopes that are responsible for eliciting allergic reactions or are the target of immunochemical detection methods. In addition to changes in allergenicity, processing procedures affect the extractability, solubility, and analysis of food allergens. Studies indicated that moist heat usually reduces the allergen reactivity of food proteins by changing protein structure, alteration of IgE binding conformational epitopes and increased digestibility. In contrast, dry heat such as baking of wheat flour and roasting of nuts most often leads to formation of new epitopes (neo-allergens) via Maillard reaction and reduction of digestibility resulting in increased allergenicity. Reduction of allergenicity by non-thermal techniques is attributed to conformational change of protein with enhanced susceptibility to proteolysis. High pressure followed by enzymatic hydrolysis appears to be one of the effective approaches in minimizing allergenicity of many foods. Microbial fermentation and selective enzymatic hydrolysis can break the sequence of linear epitopes and extensively reduce the allergenic potential of dairy proteins.

In conclusion, increased understanding of the impact of various processings on structure, digestibility and allergenic consequence of food allergens could be applied at industrial level to develop novel processing strategies aimed at reducing the prevalence of food allergies.

Impact of food proteins on gut microbiota and immune response in a mouse model of allergy with increased intestine sensitivity

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Aim: The aim of the study was to characterize gut microbiota and immune response in mice with β -lactoglobulin (β LG) induced allergy and intestine sensitivity.

M&M: Balb/C mice were fed intragastrically with β LG from cow milk throughout the experiment (21 days). Animals were divided into four groups: G11 and G14 were intraperitoneally sensitized with β LG (day 1, 7 and 14), whereas G1 and G6 groups did not undergo this procedure; induction of intestine sensitization (day 15?) was carried in groups G14 and G6 by giving DSS.

Results: Induction of intestine inflammation (G6) significantly increased level of *Lactobacillus*, *Clostridium coccoides*, *C. leptum*, *Bacteroides-Prevotella Porphyromonas* (BPP), and total bacterial number (TBN) comparing to G1 group. Similar relation for BPP and TBN was observed for β LG-sensitized groups (G11 vs. G14) groups. Qualitative analysis revealed that only *Lactobacillus* populations were affected by DSS treatment (G6 and G11). Analysis of caecal SCFA showed a significant decrease in butyrate concentration in groups G6, G11 and G14 comparing to G1. The expression level of genes coding IL-1 β , IL-4, IL-6, IL-8, IL-10, TNF α , INF- γ , MCP-1 and TLR4 was unchanged upon intraperitoneally given β LG and/or DSS. A significant decrease of IL-2 and TLR-2 gene expression was observed after intestine sensitization (G1 vs. G6). Flow cytometry analyses showed twice higher CD4+ population in the peripheral blood lymphocytes population in groups not treated with DSS. The induction of intestine inflammation increased the level of regulatory cells CD4+CD25+Foxp3+ and production of INF- γ by CD4+CD25+, and decreased IL-10 secreted by DC4+DC25+ lymphocytes. Similar distribution of lymphocytes was determined for cells isolated from head and neck, spleen and mesenchymal lymph nodes.

Conclusion: the characterized animal model of allergy with induced intestine inflammation will allow for assessment of an influence of food components on immune system and composition and metabolic activity of intestinal microbiota.

Immunoreactive properties of α -casein and κ -casein - ex vivo and in vivo studies

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Cow milk protein allergy is the most common type of food allergy in children, affecting 2-5% children under 3 years of age. The main subclass of milk proteins are whey proteins and caseins which accounting 80% of all bovine milk proteins. Caseins are the major milk allergens and are responsible for the strongest systemic allergic reactions in neonates and children.

The aim of the study was to determine the ability of lymphocytes isolated from the mouse spleen to induce an immune response with the use of the α -casein and κ -casein fractions in ex vivo and in vivo experiment model.

During the first part of ex vivo experiment the lymphocytes were isolated from the spleen of the naive mice. Lymphocytes were cultured and stimulated with α -casein or κ -casein, After 120 hours of incubation lymphocytes with allergens, the proliferation index was evaluated by using MTT assay and CFSE staining.

The second part of experiment was done in vivo animal model. Mice Balb/c were sensitized by intraperitoneal injection of a mixture 100 μ g α -casein or κ -casein with Freund's adjuvant on 0, 7, 14th day of experiment. From 48th day to 63rd day mice were intragastric fed with 200 μ L of α -casein or κ -casein (1mg/mL). The specific IgA and IgG antibodies were determined in feces and blood from 14th day of experiment, once a week. On 63rd day of the experiment mice were terminated. Lymphocytes were isolated from spleen. The lymphocytes were phenotypically labeled for CD4, CD8, CD11, CD25, CD3, CD3, Foxp3. The level of secreted cytokines in the medium and lymphocyte index proliferation was measured.

The obtained results confirmed the immunogenic potential of alpha and kappa caseins. In vitro study, index proliferation of splenocytes stimulated κ -casein was higher than α -casein in both MTT test and CFSE staining. The level of specific antibody class A in fecal sample was similar in both groups of mice. In the case of serum IgA and IgG, a higher level was observed in mice immunized with α -casein. The level of cytokines secreted by splenocytes indicates the dominance of the Th2 immune response. As a result of obtained data α -casein and κ -casein have different effect on immune response of the organism and activation of lymphocytes.

Generation and characterization of two *in vitro* systems of bone marrow derived mast cells modeling mucosal and connective tissue subsets

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BACKGROUND: There is a need for reductionist systems as tools to study the contribution of different mast cell subsets to aspects of food allergy. From *in vivo* studies, we know that different subsets of mast cells (MCs) are critical to food-induced gastrointestinal symptoms and systemic anaphylaxis. Our aim was to generate and characterize two *in vitro* systems in which MCs are developed from bone marrow progenitors modeling the two major MCs subsets involve in food allergy, from connective tissue (CTMCs) and mucosa (MMCs).

METHODS: We grew bone marrow in the presence of IL-4, IL-3 and SCF to generate CTMCs, and TGF- β , IL-9, IL-3 and SCF to generate MMCS. Mast cell protease and TLR expression was analyzed by qPCR. Measurement of degranulation was monitored by β -hexosaminidase release and cytokine production by ELISA.

RESULTS: After 4 weeks of culture and in agreement with the mast cell protease profile obtained previously from *in vivo* experiments with mice, CTMCs expressed elevated levels of MMCP-5, 6, 7 and CPA, while MMCs had a dominant expression of MMCP-2 and also expressed MMCP-1. CTMCs and MMCs differed in growth rate, toluidine blue staining and sensitivity to degranulate in the presence of compound 48/80, being CTMCs slightly more sensitive than MMCs. Both subsets responded to IgE-mediated activation with signaling, degranulation, and inflammatory cytokine release, although IL-10 was restricted to CTMCs and IL-17 was uniquely expressed by MMCs. CTMCs and MMCs showed a similar TLR expression profile, dominated by expression of TLR4 and TLR6 and both subsets were responsive to LPS but not poly(I:C). CTMC and MMC express receptors for IL-33 and TSLP, and respond to these cytokines alone or with modified activation in response to IgE cross-linking.

CONCLUSION: In summary, this data highlight the functional differences between MC subsets depending on the immune microenvironment, and provide useful protocols for *in vitro* modeling of MC subsets.

2S Albumins are involved in Cross-reactivity between Pistachio and Cashew Nut in two children

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Background. Despite cross-reactivity between pistachio and cashew nut has been clinically described, little is known about which are the allergens involved. It has been reported several allergens from these two nuts and among them, the 2S albumins Pis v 1 and Ana o 3, have been described as important allergens in allergic process; however, the relation between these 2S albumins has not been studied yet.

Aims. To evaluate IgE-mediated sensitivity to cashew nut and pistachio proteins in two children with reported allergy only to these tree nuts, identify allergens involved and analyze the IgE cross-reactivity associated.

Methods. The purification of 2S albumins from protein extracts was performed using chromatographic methods. The sequences of several peptides were done by mass spectrometry. Molecular characterization was conducted by electrophoretic methods (1- and 2-DE). Its secondary structure and thermal stability were studied by circular dichroism spectroscopy. Finally, immunoassays were performed to reveal their allergenic capacity using children's sera.

Results. Cashew nut and pistachio extracts were tested with the allergic sera by immunoblotting, revealing that both recognized mainly proteins of low molecular mass. They were isolated and purified from extracts by means of a gel filtration in Sephadex G-50 and a reverse phase in HPLC. They were identified as 2S albumins by mass-spectrometry of digested peptides (MALDI-TOF) and the sequence of several peptides were obtained. They generally show a helicoidal secondary structure, stable at 85°C, except the one from pistachio, Pis v 1, whose T_m was 60 °C. Purified proteins were recognized by the two allergic sera in immunoblotting. Moreover no IgE binding was detected in other 2S albumins from nuts and seeds. Inhibition immunoassays revealed a complete inhibition of the IgE binding to each other.

Discussion. Isolated proteins showed similar characteristics than other 2S albumins described. Moreover, the structural and phylogenetic resemblance -they share common lineal regions- between them (%I: 62) may explain the cross-reactivity between cashew nut and pistachio.

Conclusions. 2S albumins identified from pistachio and cashew nut may be involved in cross-reactivity between these tree nuts. No cross-reactivity was detected with other 2S albumins from seeds and nuts. The proteins can be used as clinical tools for in component-resolved diagnosis (CRD). This diagnosis is especially relevant having in account the severe symptoms caused by this allergenic family.

Effects of interactions with phenolic compounds on the stability of certain allergen proteins

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Recently studies on allergenic reactions to foods and prevention of allergenic reactions have been intensified. The most important reason is that a certain proportion of the population (1-3 % of adults and 4-6 % of children) is sufferer from adverse health consequences as a result of the consumption of particular foods or food ingredients. Studies have shown that the incidence of food allergies has increased in the last decade. This situation is an important challenge to both the food industry and clinically. It is known that there are non covalent or covalent interactions between phenolic compounds and proteins. The approach to these interactions generally affects the nutritional value of proteins adversely due to the modification of essential amino acids and through the inhibition of proteases. Also, the interactions may lead to change some physicochemical properties (emulsification, foaming, etc.) of food protein. However, the interactions have been considered a promising to decrease food allergenicity. Such complexation may reduce IgE binding of allergens due to irreversible precipitation, and also affects proteolysis by pepsin. Conformational structure, digestibility and aggregation are important for biological activities of dietary proteins that elicit hypersensitivity reactions in humans. Recent studies provides further evidence in support of the proposed mechanism by which phenolic compounds interactions with the food allergens contribute to its diverse biological activities and may impair antigen uptake by antigen-presenting cells. The majority of studies on the interactions between polyphenols and proteins have yielded binding constants, putative polyphenol-binding sites, the character of forces important for recognition, and effects of the complex formation on the stability and antioxidant activity of polyphenols. As a consequence, the overall aim should be an optimal design of protein-polyphenol interactions to ensure a positive contribution to food quality, protein nutrition, and decreasing food allergenicity. Also, health-related optimal dose of polyphenols to the gastrointestinal tract should be determined in future studies.

Recombinant tomato allergens: a deeply approach in the diagnostic tool

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Background: Several reports of cases of food allergy without a positive *in vitro* diagnosis test with standard extracts have driven to the identification of new allergens and initiated new studies to clarify the diagnosis of certain allergic patients. This fact occurs with tomato allergy, where two different non-specific lipid transfer proteins (nsLTPs) have been specifically identified in tomato seeds: Sola I 6 and Sola I 7, not present in the peel of this fruit where only Sola I 3 (the peel nsLTP) has been described. In this study, we analyse if intra- or inter-species cross-reactivity could be involved in the sensitizations within the same fruit and with other vegetables extracts using the recombinant form of these allergens and the evaluation by means of polyclonal antibodies (pAb) recognition.

Method: Extracts from different tomato tissues and other vegetables seeds, nuts or and a variety of purified recombinant LTPs produced in *Pichia pastoris* (Sola I 3, Sola I 6, Sola I 7, Pru p 3, and Sin a 3) were available and deeply characterized. pAb against tomato seed allergens were produced to perform the cross-reactivity assays.

Results: Recombinant Sola I 7 and Sola I 6 show a perfect IgE recognition from patient sera and same circular dichroism spectrum comparing with natural forms. *In vitro* IgG recognition of rSola I 6 and rSola I 7 to other vegetables extract and purified proteins, reveals a great cross-reactivity with Pru p 3, from peach. By contrast, no cross-reactivity is observed with Sola I 3, tomato peel nsLTP allergen, neither between them.

Conclusion: The recombinant production of new allergens is an important event in order to improve patient diagnosis by *in vitro* techniques. The results of this study with the pAbs and the production of these proteins of the same family located in different tissue of the same fruit with no IgG cross-reactivity and independent sensitization lead us to improve the patient diagnosis.

Aggregation of ovalbumin and allergenicity

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Background

Thermal aggregation is an irreversible modification of proteins with intermolecular links between unfolded proteins. Depending on the balance of attractive and repulsive interactions during heating, aggregates of various morphologies form. How different ways of aggregating ovalbumin modulate its allergenicity was investigated.

Method

An ovalbumin solution was extensively heated in two opposite electrostatic conditions to form small linear and large spherical-agglomerated aggregates. In a murine model of allergy, the Ig production when sensitizing mice with the aggregates and the subsequent elicitation phase upon an oral challenge with native ovalbumin were compared. The reactivity of specific IgE in mice sera was characterized by ELISA, Rat Basophil Leukemia assay and pepscan analysis.

Results

IgE production was significantly lower for the small than for the large aggregates, whereas IgG1 and IgG2a productions didn't change. In agreement with the IgE production, both symptoms upon oral challenge and basophil degranulation with native ovalbumin were lower for mice sensitized with the small than the large aggregates. Pepscan analysis revealed two common linear IgE-epitopes but the aggregates were similarly or differently bound and cross-linked depending on the aggregate that had been used during sensitization. These results showed that small aggregates of ovalbumin formed in repulsive electrostatic conditions displayed a lower allergenic potential than the large aggregates. The way ovalbumin aggregated also modified the IgE repertory.

Conclusion

This work illustrates links between food structure and allergenic potential on parameters from the sensitization phase with some consequences on the elicitation phase of the allergic reaction. The physicochemical conditions when heating ovalbumin and the ensuing aggregated structures are important parameters to consider in the context of allergy.

Identification of digestion resistant casein domains after *in vitro* gastrointestinal digestion followed by the incorporation of the intestinal brush border peptidases

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Background: *In vivo* studies have demonstrated that casein peptides released during gastrointestinal digestion cover a wide range of bioactive peptides and immunogenic sequences. Immunoreactive casein material has been detected in jejunum and ileum in piglets. The development of *in vitro* digestion models is of great importance to provide alternatives to animal and human experiments. However, the intestinal phase of digestion with peptidases from the intestinal brush border membrane (BBM) has been omitted in the majority of the *in vitro* digestion models developed so far. This study aimed to assess the impact of the BBM in the *in vitro* gastrointestinal digestion simulation of caseins to mimic the final digestion occurring *in vivo* in the jejunum. and identified.

Methods: The *in vitro* gastrointestinal static model harmonized within the frame of the Infogest COST Action was used and a four hours-incubation phase with BBM was added. The peptide profile was studied by HPLC-MS/MS.

Results: The incorporation of BBM affects the hydrolysis of the peptides: several were shortened by exopeptidases (amino- and carboxy-peptidases), while others are resistant to their action. From the β -casein and α 1-casein identified peptides at the end of the intestinal phase, 57% were resistant to BBMs. Twenty β -casein derived peptides were identical to those previously identified in human jejunal digests of caseins. Some of them had been previously reported as peptides with physiological activities in the organism.

Conclusions: β -casein and α 1-casein have peptides that are resistant to digestion in the gut and in some case correspond to formerly described immunoreactive domains, concretely fragments from the N-and C-terminal parts of β - and α 1-casein, being related with the most hydrophobic regions of these proteins.

Antibodies to wheat alpha-amylase inhibitor 0.19 in celiac disease

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Keywords: celiac disease, gluten-free diet, alpha amylase inhibitor 0.19, antibodies, serology, ELISA

Introduction: Cereals belong to the most important nutrients in the world even though they induce morbidity in over 2 % of the world population. The alcohol-soluble fraction of gluten (wheat-grain storage proteins) gliadins and phylogenetically related cereals' proteins induce in genetically susceptible individuals celiac disease (CLD), which is characterized by villous atrophy and crypt hyperplasia of duodenal and/or jejunal mucosa accompanied by malabsorption syndrome. Active CLD is serologically manifested by elevation of antibodies (Ab) against induction agents gliadin(s) and by autoAb against tissue transglutaminase (tTG) and endomysium. These Ab represent a diagnostic hallmark of the CLD, which is completed by histological analysis of small-intestinal biopsy in diagnostic algorithm. The sole therapy of CLD is a strict, life-long adherence to gluten-free diet (GFD) leading to healing of gut mucosa and disappearance of the serological markers. Although a long-lasting and incomplete histological recovery in CLD patients on a GFD is assumed, the histological analysis of small-intestinal mucosa is not usually performed in a follow-up of these patients.

However, gliadins are not unique wheat proteins in human pathology. Alfa-amylase inhibitors 0.19 and 0.28 (AAI 0.19, AAI 0.28) were identified as allergens. The AAI 0.19 is, moreover, capable of activating monocyte-derived dendritic cells in CLD patients via interaction with TLR4-MD2-CD14 on their cell surface. **AIMS:** We focused on analysis of AAI 0.19 role in CLD via the study of its B-cell antigenicity. To this end, we studied the occurrence of serum IgA, IgG and IgE Ab against AAI 0.19 in patients with active CLD, those on a GFD and healthy individuals. We used an immunoblot technique employing the mixture of isolated wheat AAI 0.19 and AAI 0.28, and developed a reproducible, robust, quantitative ELISA for testing serum IgA and IgG Ab with wheat recombinant AAI 0.19 as an antigen.

Results: Using the immunoblot we detected reactivity of IgA anti-IAA 0.19 and/or anti-AAI 0.28 Ab at dilution 1:500 in eight out of 32 active CLD patients, three out of 30 CLD-GFD and four out of 46 healthy controls. By this technique, the IgG Ab were detected at the dilution of 1:2500 in 14 out of 32 patients with active CLD, two out of 30 CLD-GFD patients and four out of 46 healthy individuals. Surprisingly, we also detected IgE isotype of Ab recognizing IAA 0.19 and/or IAA 0.28 in patients with 13 out of 32 CLD patients, three out of 23 CLD-GFD, and two out of 22 healthy controls. The ELISA revealed that IgA anti-IAA 0.19 Ab were significantly elevated ($P < 0.001$) even in patients with active CLD (117.2 ± 105.3 AU, mean \pm standard deviation) and CLD-GFD (80.1 ± 43.6 AU) in contrast to healthy controls (50.5 ± 24.3 AU). Although we detected reduced average level of IgA anti-AAI 0.19 Ab in a cohort of CLD-GFD in comparison with active CLD, statistically significant difference was not found. The frequency of anti-AAI 0.19 Ab seropositivity in tested cohorts was as follows: 13 out of 30 active CLD patients, 15 out of 46 CLD-GFD patients and two out of 59 healthy controls were seropositive for these Ab. IgG Ab were significantly ($P < 0.001$, 149

$\pm 78,4$ AU) elevated only in patients with active CLD, while they were significantly decreased in CLD-GFD patients ($P < 0.001$, 59 ± 37.1) both in contrast to patients with active CLD and healthy controls (82.7 ± 33.7). Fourteen out of 30 patients with active CLD, one out of 46 CLD-GFD and one out of 59 healthy controls were seropositive for IgG anti-IAA 0.19 Ab. Interestingly, six out of 30 active CLD patients were seropositive for both isotypes of anti-IAA 0.19 Ab.

Conclusion: Our results for the first time document a robust IgA and IgG Ab reactivity against wheat non-gluten protein IAA 0.19 in a substantial number of patients with active CLD. While IgG anti-tTG Ab isotype is missing in patients on a GFD, anti-IAA 0.19 IgA Ab persisted in over 30 % CLD patients on a GFD. Our results contributed to conceiving an immunological platform for further analysis of the role of wheat AAI 0.19 possessing adjuvant and allergic properties, in development of CLD.

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Generation and characterization of a recombinant nonallergenic chicken tropomyosin as a potential molecular reference for allergenicity assessment studies.

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Background: Tropomyosins are well-known allergenic proteins identified in a wide group of invertebrate species, namely crustaceans, molluscs and insects. In invertebrates, tropomyosins are classified as panallergens, while in vertebrates they seem to be non-allergenic proteins [1,2]. Therefore, the main objective of this STSM concerned the expression of a recombinant chicken tropomyosin, as an example of non-allergenic protein, to be used as a potential molecular reference in food allergenicity assessment studies, in comparison to pre-existing recombinant preparations of well-known allergenic crustacean tropomyosins, namely of Pen a 1 (*Penaeus aztecus*) [3], Pen m 1 (*Penaeus monodon*) and Cra c 1 (*Crangon crangon*).

Methods: The chicken (*Gallus gallus*) tropomyosin alpha-1 chain isoform X1 was selected and its DNA string was cloned into pET vector. Two independent batches of recombinant His6-tagged chicken tropomyosins were expressed in different strains of *E. coli*, followed by IMAC purification and physicochemical characterisation using circular dichroism (CD) spectroscopy, and dynamic light scattering (DLS) after identity verification based on mass spectrometric analysis. IgE-binding was investigated in western blot analysis, and functional mediator release was done in humanized rat basophil leukemia (RBL) cells passively sensitized with IgE from shrimp-allergic sera.

Results: The second batch of recombinant chicken tropomyosin presented the best molecular characteristics, although both batches were useful to compare with pre-existing batches of recombinant allergenic crustacean tropomyosins. Results from CD and DLS analysis indicated the existence of the typical alpha helical secondary structure and dispersity of tropomyosins. IgE immunoblot analysis with sera from shrimp-sensitized individuals revealed minor IgE binding of both recombinant chicken tropomyosin batches. In line with this observation, specific mediator release indicated an at least 1E3- to 1E4-times lower allergenic potency of recombinant chicken tropomyosin when compared to rCra c 1, rPen a 1, rPen m 1 or shrimp extract.

Conclusion: Even though having minor IgE reactivity, the chicken tropomyosin appears to be hypoallergenic in comparison to shrimp tropomyosin. Availability of high and low IgE reactive homologous allergens, such as tropomyosins, may allow calibrating biological assays for allergenic risk assessment of novel proteins.

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Comparison of the digestibility and antigenicity of raw and roasted whole peanut allergens

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Background

Process induced physical-chemical modifications affect the allergenicity of peanuts to some extent. Several factors, including the food matrix, the process parameters and the inherent biochemical properties of allergens, may influence the stability of allergens to proteolytic degradation. To this purpose, many studies have attempted to assess the stability of allergens in processed food products by mimicking the physiological steps of digestion.

The digestion stability of peanuts has been investigated only for purified allergens, neglecting the relevant matrix effects that occur both in raw and processed foodstuff. In this work, we determined the stability of peanut allergens in the whole food matrix, with the aim of refining the knowledge about peanut allergenic determinants.

Method

Gastrointestinal digestion of raw and roasted peanuts was carried out using the harmonized *in vitro* static digestion models (Minekus et al. 2014), coupled to proteomic and antigenic characterization of the digests. The gastrointestinal digestion was completed with porcine brush border membrane enzymes (BBM) to simulate the jejunal degradation. Proteins and peptides 2 survived to digestion were characterized by proteomic analysis (SDS-PAGE, RP-HPLC and LC-MS/MS) and their allergenicity was investigated by Rat Basophilic Leukemia (RBL) cells assay.

Results

Fragments of Ara h3, Ara h2 and Ara h6 detected by SDS-PAGE were found resistant to digestion either in raw or roasted peanuts. A large number of digestion stable peptides (mainly from Ara h3) was identified by LC-MS/MS analysis. As a general features, roasted peanuts were more digestible than the raw counterpart, as also confirmed by RP-HPLC. Consistently, RBL assay highlighted that the roasting process reduces the allergenic potential of peanuts.

Conclusions

These findings provide new and more realistic insights on digestion stability of peanut allergens and suggest technological strategies to reduce their allergenic potential.

The effect of *Akkermansia muciniphilia* on house dust mite induced allergic airway inflammation

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Background

Akkermansia muciniphilia is among the most abundant bacterial species in the gut of healthy humans. Despite multiple health benefits have been associated with *A. muciniphilia*, its molecular mode of action is not yet well-understood. The aim of this project was to investigate the mode of action of *A. muciniphilia* in a murine model of house dust mite (HDM) induced allergic airway inflammation. In particular, we aimed to understand whether the effect is mediated via toll like receptor (TLR) signaling, since it was recently demonstrated that a specific outer membrane protein of *A. muciniphilia* can interact with the host TLR2.

Method

To investigate the effect of *A. muciniphilia*, live or non-viable heat treated bacteria were administered daily by gavage in B6 wild type or TLR signaling deficient (Myd88^{-/-}) mice five days prior to and concurrent with induction of allergic airway inflammation by nasal administration of HDM. Airway inflammation was evaluated by total and differential cell counts in bronchoalveolar lavages. Mucosal T helper cell, regulatory T-cell and dendritic cell subsets were analysed by flow cytometry.

Results

Oral administration of both live and heat-treated *A. muciniphilia* did reduce HDM induced allergic airway inflammation in both wild type and TLR signaling deficient mice. Heat-treated *A. muciniphilia* was found to have a more potent effect compared to live bacteria. Analysis of mucosal cell subsets by flow cytometry indicated that the effect of *A. muciniphilia* involves increased expression of the lymph node homing factor C-C chemokine receptor type 7 (CCR7) on lung dendritic cells.

Conclusion

The results indicate that the anti-inflammatory effect of *A. muciniphilia* is not primarily TLR signaling mediated and does not require the bacteria to be viable. Further studies are needed to understand the interplay between *A. muciniphilia* and host immune regulation, but CCR7 expression of dendritic cells might play an important role.

Investigation of clinical and immunological reactivity of the major fish allergens parvalbumins

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Background: Fish allergy affects up to 2% of the world population and often causes severe life-threatening reactions. Major fish allergens parvalbumins (PVs) occur in two evolutionary sublineages, α and β , with bony fish β PVs being generally allergenic. Great diversity among fish PVs presents a challenge in establishing diagnostic methods and exploring the cross-reactivity among different fish species. Calcium-binding regions of PVs play a critical role in their IgE reactivity and a hypoallergenic derivative of carp parvalbumin with mutated calcium-binding sites has been developed for specific immunotherapy of fish allergy.

Methods: We investigated the immunological and functional reactivity of 11 PVs from different sources (bony fish β PVs; cartilaginous fish α PVs; PVs from frog and chicken and the mutant PV from carp) with 15 fish allergic subjects. Specific IgE antibody was quantified by ELISA and clinical and functional reactivity analysed by skin prick test (SPT) and basophil activation test (BAT), respectively.

Results: β PVs showed higher allergenic activity in all three diagnostic tests (ELISA, SPT and BAT) compared to the α PVs. PV-specific IgE, commonly used as a diagnostic marker for fish allergy, correlated poorly to the IgE cross-linking and degranulation capacity in BAT. Mutant carp PV showed reduced IgE reactivity and capacity to activate basophils when compared with natural β PVs, with only 20% of the patients reacting to the mutant PV as compared to 60-90% reacting to natural β PVs in ELISA and BAT

Conclusion: We demonstrated that BAT has the potential to confirm true fish allergy, predict cross-reactivity of PVs and can possibly replace oral food challenges in patients with inconclusive SPT and/or ELISA tests. Furthermore, this study highlights the possible importance of conformational IgE epitopes of PV allergens for inducing allergic reactions and confirms the effectiveness of the hypoallergenic PV mutant.

Food allergy skin sensitization: A comparative study with three different gluten products in Brown Norway rats

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Background: Allergy is a multifactorial disease which can affect different organs. It has been shown that skin sensitization in mice could trigger food allergy. Recent data suggest that the skin could be the first step to food allergy. Wheat gluten and particularly the hydrolyzed forms of gluten have been incriminated in both food and skin allergies. Wheat is a major food component of western diet and hydrolysis of wheat gluten is widely used in order to increase the solubility and to facilitate its incorporation in food products. Hydrolyzed wheat proteins have been reported to cause allergic reaction in wheat tolerant patients after application of cosmetic products containing hydrolyzed wheat.

Methods: In an animal model of food allergy skin sensitization, three forms of gluten (unmodified gluten, acid hydrolyzed gluten and enzyme hydrolyzed gluten), were investigated to decipher the impact of skin sensitization on the intestinal immune response. To do so, Brown Norway rats bred on a gluten-free diet were sensitized three times a week during five weeks by an application of gluten on their slightly damaged abdominal skin.

Results: We demonstrated that the three forms of gluten were able to induce food allergy, as all the sensitized rats display elevated levels of specific IgE and IgG1 and presented an increased ear thickness in an ear-swelling test. Moreover, we observed that the three forms of gluten induced an immune response both in the abdominal skin and in the intestinal tissue. Indeed, all sensitized rats displayed an increase of CD4+T cells within the lamina propria compared to control rats.

Conclusion: Using a model of food allergy skin sensitization, we proved that skin sensitization is sufficient to induce a food allergic like response in the intestine. This work may lead to the development of atopic march model combining skin and food allergy.

Allergen identification in two main Mediterranean fish species using proteomics

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Background: Food allergies are a common health problem around the world. Ninety percent of allergies are accounted to the big eight food products, being fish one of them. Fish is becoming an important alternative for meat products due to its high nutritional value and healthy flesh. Unfortunately fish can cause adverse immune-mediated reactions in some individuals. This allergic reaction to food, affects 1-3% of the population. Parvalbumin is the main fish allergen, a small and highly stable calcium-binding muscle protein. Other fish allergens have been identified as well – such as enolases, aldolases or fish gelatin – but their importance has been only shown for a limited number of fish species. While cod allergens have been identified in detail, the identification in species like *Sparus aurata* (gilthead seabream) and *Dicentrarchus labrax* (European seabass) has not yet been addressed.

Objective: Identification of the IgE reactive proteins in the two Mediterranean species.

Methods: Muscle protein extracts were prepared from cod, gilthead seabream and European seabass. Sera from fish allergic patients was used to identify IgE reactivity on line blots. Fish proteins were labeled with fluorescent dyes and separated on 11 cm IPG bluestrips with pH 3-10 by 2-dimensional (2D) SDS-PAGE using a multiplex flatbed system. Sera from positive reactions on the line blots were used as a pool on 2D immunoblots. IgE-reactive proteins were identified using MALDI-TOF/TOF. Skin prick tests were performed on fish allergic patients.

Results: Positive IgE-reactivity in the line blots to parvalbumin-like bands was shown in 5 out of 14 patients. The three fish extracts showed various protein bands in a range from 6-70 kDa and various protein spots in the chosen pH range. Identification of positive IgE-reactive proteins showed parvalbumin, enolase and aldolase. Proteins from the two Mediterranean fish species gilthead seabream and European seabass seemed to be similar to cod allergens but further investigation is still in progress.

Conclusions: Allergenicity of different fish species might be variable for sensitized patients. The characterization of allergens from the two species in this study will need to be addressed in future studies.

Keywords: Fish allergens, IgE-reactivity, proteomics, immunoblots

Immunoproteomics characterization of allergenic and non-allergenic tropomyosin orthologs

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Background

The digestion pattern of proteins plays a key role in allergenicity. If the enzymatic digestion is not efficient, bigger peptides/proteins can persist and cause sensitization. Tropomyosin represents the major allergen of crustaceans, which is a highly conserved protein present in muscle cells of vertebrates and invertebrates. The hypothesis of the current work is that shrimp tropomyosin (TM) is not fully digested or exhibits a digestion pattern that generates some peptides that can be immunogenic. To assess this hypothesis, both purified and recombinant chicken and shrimp TM orthologs were in vitro digested and analyzed for their degradation and immunoreactivity pattern.

Methods

One milligram of both shrimp and chicken TM orthologues has been processed through simulated oral, gastric and intestinal digestion using INFOGEST protocol. The cleavage pattern was subsequently analysed by proteomics and immunoproteomics using 1D and 2D tricine gel electrophoresis coupled with mass spectrometry. The immunoreactivity pattern was evaluated using 1D immunoblotting against serum (IgE and IgG) of patients allergic to shrimp TM (allergen Pen a 1).

Results

2D Tricine-SDS-PAGE analysis revealed that chicken TM ortholog was sensitive to proteolytic activity during stimulated gastric digestion with pepsin at low pH, in contrast to shrimp TM ortholog that was not cleaved. These results were consistent with the immunoreactivity assays, which demonstrated a high reactivity of both IgE and IgG against shrimp TM after oral and gastric digestion and no reactivity against chicken TM.

Conclusion

The resistance of shrimp TM ortholog to gastric simulated digestion supports the hypothesis that undigested proteins may enhance the possibility of sensitization process. Considering the importance of protein cleavage before absorption it can be concluded that simulated gastric digestion coupled to the used immunoproteomics approach could represent a starting point for the evaluation of allergenicity of novel foods.

Keywords

Tropomyosin, digestomics, immunoproteomics, allergenic peptides.

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Lupine allergens in food products: a new real-time PCR approach for its detection and quantification

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Background: Lupine is widely used as a functional food and as an ingredient in all kind of food products. However, it is also a source of allergens capable of inducing allergic reactions in sensitised individuals, representing a potential risk even with the ingestion of trace amounts. Thus, this legume seed was recently included in European Union regulations as a food whose presence must be declared and highlighted in the list of labelled ingredients of pre-packaged foods, regardless of their amount [1]. To help food industry in allergen management, and to guarantee life quality of sensitised individuals, the development of analytical methods able to detect and quantify lupine allergens is of great importance.

Methods: A real-time PCR approach with a TaqMan™ probe was proposed to detect and quantify lupine as a potential hidden allergen in food products. Reference mixtures of known quantities (10% to 0.0001%) of *L. albus* in rice and wheat flours (with and without baking) were prepared. A normalised calibration model was developed targeting an encoding sequence of the PR-10 protein of *L. albus* and an eukaryotic reference gene.

Results: Model mixtures of lupine in rice presented the best sensitivity results, suggesting that the performance of the method is affected by food matrix and baking. The real-time PCR assay showed absolute and relative sensitivities of 1 pg of lupine DNA (1.7 DNA copies) and 0.0005% (w/w) of lupine in rice, respectively. Results of normalised and non-normalised models were compared. The normalised calibration model based on the ΔC_t method, in the range of 10-0.0005%, exhibited the best performance parameters, being successfully validated. From the 27 tested commercial foods, it was possible to quantify lupine in 5 samples that were in compliance with labelling.

Conclusion: A useful and accurate approach was proposed for lupine detection and quantification in food products, to verify labelling compliance and to guarantee consumer's protection.

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Detection of soy (*Glycine max*) allergens in processed model and commercial foods by ELISA techniques: Influence of selected target protein and ELISA format applied.

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Soy (*Glycine max*) is included in the list of allergenic food of mandatory labelling in the current European regulation. Soy proteins are widely used in the food industry due to their functional properties and competitive price, so they are often present as hidden allergens due to cross-contamination.

The aim of this study was to develop ELISA techniques to detect soy in food. These techniques are based on the determination of two target proteins (native or heat denatured forms of glycinin or native β -conglycinin) and using two formats (indirect competitive or sandwich for glycinin). These techniques have been applied to detect soy in model processed foods subjected to pasteurization (sausage), baking (bread) or sterilization (pate) as well as in commercial food.

To perform this work, glycinin and β -conglycinin were purified from soybeans using isoelectric precipitation, salting-out and size exclusion chromatography, and glycinin was subjected to heat treatment for denaturation. Antisera were obtained in rabbits and specific antibodies purified by immunoabsorption and labelled with peroxidase.

Results obtained showed that the sandwich format for both, glycinin and β -conglycinin, could detect the addition of 0.005% and 0.05% of soy (w/w) in sausage and bread, whereas they could not detect soy in pate even at the percentage of 1% of added soy. The competitive format for glycinin showed a lower sensitivity than the sandwich format detecting percentages of 0.25% in sausage and 0.5% in bread. The sandwich ELISA based on the determination of heat denatured glycinin could detect similar percentages of soy than the sandwich ELISA for the native protein in sausages and bread and was the only technique able to detect soy in pate at 0.1% addition.

These results show that the particularities of the target protein and the ELISA format as well as the heat processing applied to food influenced the determination of allergenic proteins present in food.

Participants

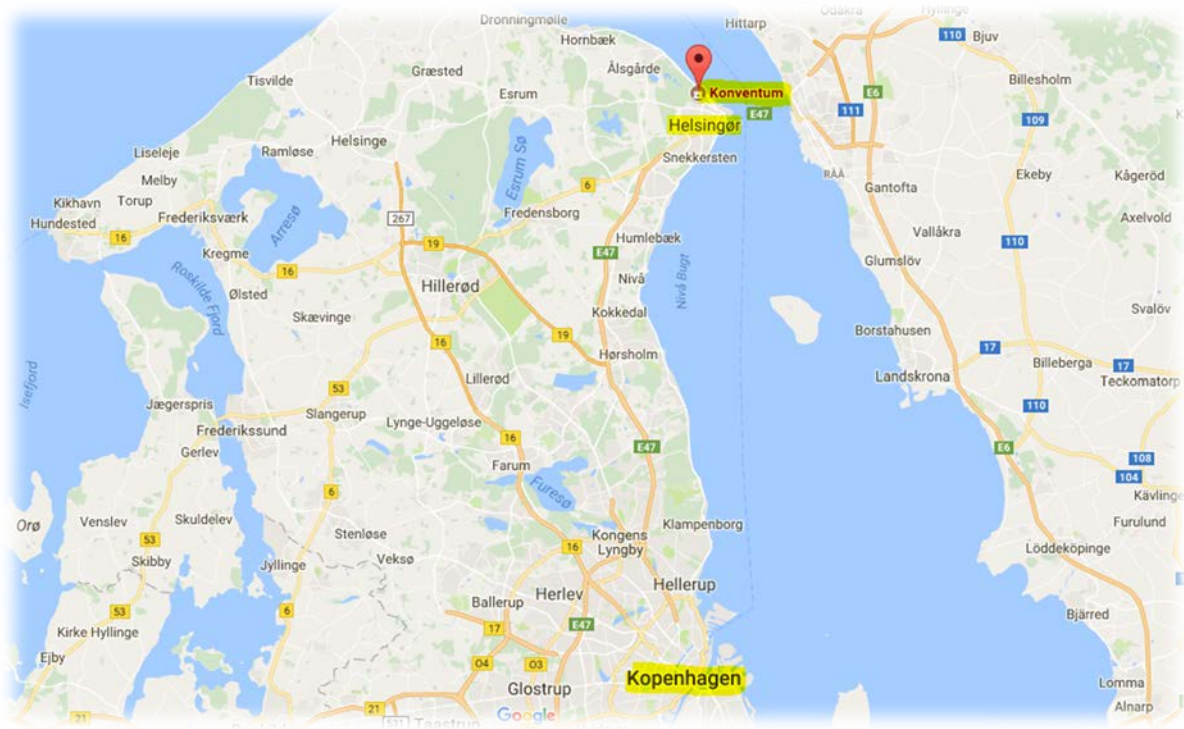
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Local Information

Conference will be held in :

Konventum
Gl Hellebækvej 70
3000 Helsingør
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Telephone +45 49 28 09 00
www.konventum.com



How to reach Helsingør:

The Konventum can be reached via train from airport Copenhagen.

Venue: Conference Center Konventum, Gl Hellebækvej 70, 3000 Helsingør, Denmark

www.konventum.com

Train from Copenhagen airport every 20 min. Bus no 803 from Helsingør station to Konventum

Total travel time to Konventum approx. 1 h 30 min. Timetable at www.rejseplanen.dk