



# ***SPEAKER ABSTRACTS***

## **SYMPOSIUM ON SENSITIZING PROPERTIES OF PROTEINS**

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**Sponsored by:**

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## **SESSION I: FOOD-SPECIFIC FACTORS**

### **ABSTRACTS**

#### **Prevalence of allergies around the globe: the big eight (ten? thirteen?)**

*Montserrat Fernández-Rivas, MD, PhD (Hospital Clínico San Carlos, Madrid, Spain)*

#### **Variability of individual protein contents in crops**

*Gregory S. Ladics, PhD, DABT, ATS (DuPont Agricultural Biotechnology, Wilmington, DE, USA).*

#### **Food processing and the food matrix – effects on the allergenicity of foods**

*Professor Clare Mills (University of Manchester, United Kingdom)*

#### **Matrix effects on allergenicity**

*Christal Bowman, PhD (US Environmental Protection Agency, Research Triangle Park, NC, USA)*

#### **Allergen-specific pattern recognition receptor pathways**

*Marsha Wills-Karp, PhD (Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA)*

#### **Danger signals from allergens: proteolytic action**

*Ronald van Ree, PhD (Academic Medical Center, Amsterdam University, The Netherlands)*



## **Prevalence of allergies around the globe: the big eight (ten? thirteen?)**

*Montserrat Fernández-Rivas, MD, PhD (Hospital Clínico San Carlos, Madrid, Spain)*

It is a “classic” in food allergy that “eight types of foods account for 90% of food-allergy reactions: cow’s milk, eggs, peanuts, tree nuts, fish, shellfish, soybeans, and wheat.” They are known as the big eight, and come from studies performed in the USA, but when surveys were performed in other continents, new and challenging data appeared. Milk and egg are the most prevalent foods worldwide in small children provided they follow a westernized diet. Peanut allergy is prevalent in countries with a westernized lifestyle and virtually absent in Asia and Africa despite a high consumption. Tree nuts are also prevalent in Europe with hazelnut at the top in patients allergic to birch pollen, whereas walnut is important in the Mediterranean patients allergic to lipid transfer proteins. The prevalence of soybean and wheat allergy was very low in the EuroPrevall surveys, but they are important food allergies in Japan. Fruits (such as peach, apple, kiwi, and melon), seeds, and vegetables (such as celeriac, carrot and tomato) are amongst the most prevalent foods inducing allergic reactions across Europe, can be severe, and are not in the big eight. The big eight concept has been of help to the scientific community and policy makers in the field of food allergy, but deserves a revision and geographical adaptation in the light of our current knowledge of food allergy epidemiology.



## **Variability of individual protein contents in crops**

*Gregory S. Ladics, PhD, DABT, ATS (DuPont Agricultural Biotechnology, Wilmington, DE, USA).*

Comparisons of food allergen levels across different cultivars of a non-GM crop have rarely been attempted because protein thresholds for eliciting an allergic reaction have typically not been determined and vary by individual. Furthermore, since allergic individuals attempt to avoid offending foods completely, development of methods to quantify allergen levels in new non-GM cultivars hasn't been considered necessary to protect public health. There is also no clear relationship between greater exposure to a protein by individuals and the frequency of allergy within populations. Thus, quantitative analytical methods designed to measure most allergens are lacking. Where endogenous allergen levels have been evaluated across non-GM crops, they vary considerably. In addition to cultivar differences, natural variability in allergen levels can occur in response to differing environmental conditions, harvest timing, or storage conditions. Nevertheless, some regulatory authorities require the assessment of endogenous allergen levels in certain GM crops (e.g., soybean) despite a lack of knowledge on natural variability in non-GM crops. Objectives of this presentation are to 1) discuss why quantifying levels of endogenous allergens in GM crops is of negligible value in the safety assessment; and 2) provide data on variability of allergen levels in non-GM soybean using absolute protein quantification by mass spectrometry.



## **Food processing and the food matrix – effects on the allergenicity of foods**

*Professor Clare Mills (University of Manchester, United Kingdom)*

Food processing has been used by humankind since ancient times to preserve foods, making certain types of foods safe to eat by inactivating toxic and anti-nutritional factors, as well as enhancing their palatability. Classically, food processing has involved the application of physical (such as heating), chemical (such as pickling and smoking), and biological (fermentation) processing procedures, alone and in combination. Such conditions can result in chemical modification of the biopolymeric and other components of raw ingredients (for example, modification by sugars through the Maillard reaction) and disruption of naturally occurring food structures (such as cellular structures found in animal and plant tissues, casein micelles, and fat globules). In addition the processing procedures result in rearrangements of polymers to form new structures – such as the foam structure of whipped egg white or the gelled structure of yoghurt.

Investigating the effects of processing on allergenicity is difficult because the protein fraction of processed foods is usually insoluble, and hence not tractable to the types of studies usually undertaken to characterise allergenic molecules. As a consequence, research has generally been focussed on residual soluble protein fractions from processed foods. Despite such limitations, it is emerging that processing induced modifications of food proteins can modulate their allergenic activity both by modification of IgE-binding epitopes or potentially through the formation of neo-epitopes. Furthermore, certain potent food allergens with a conserved disulphide skeleton belonging to the prolamin superfamily appear to retain their structure and solubility after food processing as well as their IgE reactivity. This includes the allergenic lipid transfer proteins, such as Pru p 3 and the 2S albumin allergens such as Ara h 2/6. Only after severe heat treatment (extended heating times or conditions used in processes such as canning) do they become unfolded and have reduced IgE reactivity. Such effects may explain why heavily processed milk proteins can be consumed by children whose cows' milk allergy is in the process of resolving.

In addition to modification of epitopes, the food matrix itself may affect the allergenicity of foods by modifying the bioaccessibility of allergens in the gastrointestinal tract. Studies of the digestibility of allergen molecules such as Pru p 3 in natural food matrices show that the molecules are protected by other food constituents from thermal denaturation, and their resistance to digestion in whole foods is enhanced. Thus, the food matrix may potentially enhance the release and stability of allergens in processed foods, explaining why certain foods, such as confectionary, may present more of a risk of eliciting a severe reaction than others. Data on such aspects are needed to inform the allergenic risk assessment process and to identify if there are key processing conditions or types of food matrix which may be able to either reduce or enhance the allergenic potential of foods.



## **Matrix effects on allergenicity**

*Christal Bowman, PhD (US Environmental Protection Agency, Research Triangle Park, NC, USA)*

Foods consist of a complex mixture of substances collectively known as a food matrix. Multiple guidance and consensus documents for assessing potential allergenicity cite the need to consider the effects of the food matrix. However, information regarding the role of the matrix in allergenicity is scarce. Currently, the primary influences of the matrix on allergenicity are thought to be antigen bioavailability/release, digestibility, and interactions with the immune system. Particular food matrix structures, such as those in oilseeds, can delay the release of antigen after ingestion. Digestibility, assessed *in vitro* and *in vivo*, is likely to vary depending on protein form (purified vs. present in a matrix). Certain matrices may exert adjuvant effects (e.g., peanut extract). Lipid content can influence sensitization and the severity of elicitation responses, via altered antigen release/digestion or immunomodulation. Matrix sugar content can affect modification of allergens during processing, enhancing or diminishing allergenicity. Other considerations include altered antigenicity due to protein interactions, and matrix-related thermostability. Additionally, matrix issues confound measurement of proteins in foods, limiting our understanding of exposure. The ability to conduct risk assessment with respect to potential allergenicity is complicated by food matrix issues, and thus will require further research in this area.

*\*The views expressed in this abstract are those of the author and do not necessarily represent the views or policies of the US Environmental Protection Agency.*



### **Allergen-specific pattern recognition receptor pathways**

*Marsha Wills-Karp, PhD (Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD, USA)*

Allergic diseases continue to plague modernized societies, underscoring the need to identify the molecular basis for the propensity of a small number of environmental proteins to provoke maladaptive, allergic responses. Recent data suggest that the ability of allergenic proteins to drive allergic responses in susceptible hosts is driven by their unique innate immune activating capabilities. Although the identification of allergen-specific pattern recognition receptors is in its infancy, studies to date have shown that allergens drive Th2-biased immune responses via directly engaging C-type lectin receptors (dectin-2, DC-SIGN, and mannose receptor) on dendritic cells and/or mimicking toll-like receptor 4 signaling complex molecules expressed on airway structural cells. Elucidation of the specific innate immune pathways activated by allergens holds great promise in defining new therapeutic targets for the treatment of allergic diseases.

*(See “Resources” tab for more information.)*



## **Danger signals from allergens: proteolytic action**

*Ronald van Ree, PhD (Academic Medical Center, Amsterdam University, The Netherlands)*

“What makes an allergen an allergen?” has been the subject of debate for several decades. Most important allergens known to date come from a limited number of protein families, when clustered based on their structure. Some functional properties of proteins have been proposed as being decisive for possessing allergenic properties. For example, many known allergens have been shown to have the ability to bind lipophilic structures, such as the lipid transfer proteins, the lipocalins (amongst which include major pet allergens) and the major house dust mite allergen Der p 2. In particular, the binding of LPS to Der p 2 has attracted a lot of attention over the past years. Another property that has drawn attention as a potential factor in allergenicity is the enzymatic activity of proteins. Proteolytic activity is mentioned most often as a pro-allergenic property, in particular the activity of cysteine proteases in house dust mites. The most prominent representatives of this group are Der p 1 and Der f 1. Proteolytic activities have been implicated in breaking the epithelial barrier through loosening tight junctions, and in directly favoring IgE production by skewing the immune response towards Th2. Also for some pollen allergens it has been described that they can break the epithelial barrier.



## **SESSION II: EXPERIENCES FROM THE HUMAN FOOD CHAIN – WHAT CAN BE LEARNED FROM THE EPIDEMIOLOGICAL AND CLINICAL STUDIES?**

### **ABSTRACTS**

#### **Describing patterns of IgE sensitization to molecules using modern technologies**

*Adriano Mari, MD (Allergome - Allergy Data Laboratories sc, Latina, Italy)*

#### **New proteins in the food chain: Is there evidence of new sensitization and allergies?**

*Richard E. Goodman, PhD (FARRP, University of Nebraska – Lincoln, NE, USA)*

#### **Digestibility and sensitization**

*Isabella Pali, Dr. rer. nat., Dr. scient. med. (Medical University of Vienna, Austria)*

#### **Factors modifying allergen sensitization and predisposition to allergic diseases**

*Simon P. Hogan, PhD (Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA)*



## **Describing patterns of IgE sensitization to molecules using modern technologies**

*Adriano Mari, MD (Allergome - Allergy Data Laboratories sc, Latina, Italy)*

Testing for allergy in IgE-mediated diseases changed dramatically in the last ten years. The over centenary in vivo testing using allergen extracts and skin test is going to be replaced by the use of products and means from modern technologies, namely allergenic molecules, micro-technologies, and ICT including bioinformatics. This combination is leading to totally different approaches in research, epidemiology, diagnosis of allergic diseases, and is better driving decision-making in drug and immune-therapy and environmental exposure monitoring and control.

We recently reported in several published studies how all this is possible in real life, including the chance for creating real time repositories of data on global scale at no additional costs rather than affordable diagnostic and research expenses. Examples are studies using routine diagnostic data, documenting the IgE reactivity to panels of homologous molecules, reporting IgE testing for new allergens, hypoallergens, and for compounds with unknown IgE reactivity. Information will be given on tools developed by us and supporting our current and future studies, namely the *Allergome* platform and its modules, *ReTIME*, *InterAll*, and *AllergomeConsumer*.

We are confident that the new proposed strategies for describing patterns of IgE sensitizations will be advantageous for patients, allergists, authorities, and companies in the field of allergy and related-fields.



## **New proteins in the food chain: Is there evidence of new sensitization and allergies?**

*Richard E. Goodman, PhD (FARRP, University of Nebraska – Lincoln, NE, USA)*

We have extensive knowledge of allergy to many foods and sensitization patterns (skin prick tests and specific IgE) to dietary proteins. However, IgE and SPT data provide poor correlation with symptom induction, possibly due to low exposure in food, processing (denaturation), rapid digestion or lack of multiple epitopes that bind IgE with high affinity. Evidence may be gained by pre- and post-introduction monitoring in specific consumer groups, but foods are complex (e.g., kiwi) with variable protein content. Examples of food introduction and development of corresponding allergies in young children are sometimes clear. But characterization of specific sensitization initiation is rarely possible. The route of exposure and involvement of other dietary components are often unknown. Some food allergy may occur due to dermal or airway exposure, and cross-reactivity of proteins of high amino acid sequence identity and structural similarity may lead to some food allergies. Some have claimed genetically modified (GM) soybeans are responsible for apparent increases in allergy to soy; yet tests of IgE binding with sera from soy allergic subjects from different populations failed to implicate the introduced protein CP4 EPSPS (Hoff et al., 2007). There is little evidence that novel proteins used as food ingredients cause sensitization.



## **Digestibility and sensitization**

*Isabella Pali, Dr. rer. nat., Dr. scient. med. (Medical University of Vienna, Austria)*

The stability of antigens against different treatments (heating, cooking, acidic/basic solutions, enzymes) has long been acknowledged to be important for their sensitization capacity. Stability against digestion is regarded as being of particular significance. Several studies have shown that the ability of a food protein to survive the gastrointestinal passage in a structurally intact form can increase its potential to act as allergen. Accordingly, if the physiological digestion is impaired, food antigens, which would normally be easily broken down by digestive enzymes and ignored or tolerated by the immune system, could gain sensitizing capacity. Such a situation of impaired digestion could result from intake of acid-suppressing medication, used for treatment of gastrointestinal disorders. Indeed, different anti-ulcer drugs increased the risk for sensitization against concomitantly applied proteins in animal and human studies. These results imply that disturbance of physiological gastrointestinal digestion represents a risk factor for food sensitization, also against normally digestible antigens.



## **Factors modifying allergen sensitization and predisposition to allergic diseases**

*Simon P. Hogan, PhD (Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA)*

While the precise etiologies of allergic diseases (respiratory, cutaneous, and intestinal) are not yet fully delineated, experimental and clinical studies indicate that an aberrant CD4<sup>+</sup> T-cell response directed against allergens is pathognomonic of these diseases. Allergens activate innate immune pathways, thereby triggering epithelial cell and dendritic cell (DC) activation and subsequent pro-inflammatory cytokine and chemokine production (IL-4, IL-25 and IL-33). This leads to the development of an allergen-specific CD4<sup>+</sup> Th2/Th17 response and granulocytic inflammation. A number of factors have been implicated in allergen penetration of the mucosal surface and sensitization, including allergen-specific structural physical-chemical properties and/or host genetic modifying factors such as defects in mucosal barrier function and predisposition to atopy. In this presentation, I will discuss, compare, and contrast allergen-specific factors and endogenous pathways that influence allergen sensitization and predisposition to allergic diseases.



## **SESSION III: IN VITRO AND IN SILICO MODELS FOR SENSITIZATION**

### **ABSTRACTS**

#### **Allergenicity of linear versus conformational epitopes**

*Anna Pomés, PhD (Indoor Biotechnologies, Inc., Charlottesville, VA, USA)*

#### **Allergen protein families – what makes them so peculiar?**

*Karin Hoffmann-Sommergruber, PhD (Medical University of Vienna, Austria)*

#### **Implementation of the results from the EU Framework Programme 6 funded project Sens-it-iv**

*Dr. Erwin L. Roggen (Novozymes A/S and 3Rs Management and Consultancy, Bagsvaerd, Denmark)*

#### **Translational testing of allergenicity of proteins: from animal testing to *in vitro* testing with mouse intestinal organoids**

*Joost Smit<sup>1</sup>, Marianne Bol-Schoenmakers<sup>1</sup>, Rob Bleumink<sup>1</sup>, Jean Paul Ten Klooster<sup>2</sup>, Raymond Pieters<sup>1,2</sup>*

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<sup>2</sup> Research Centre for Technology and Innovation, Utrecht University of Applied Sciences (UAS), The Netherlands.

#### **Tools and technologies for immunogenicity and allergenicity risk management**

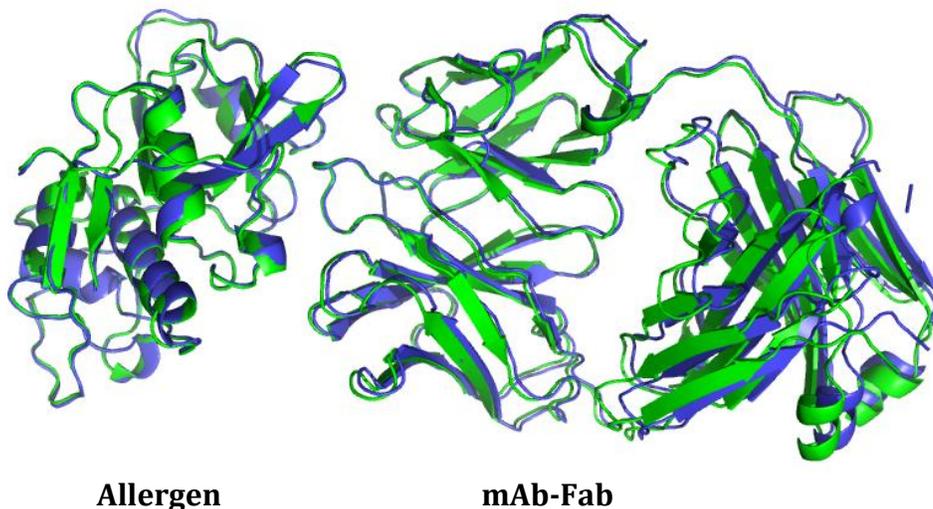
*Jeremy Fry, PhD (ProImmune Limited, Oxford, United Kingdom)*



## Allergenicity of linear versus conformational epitopes

Anna Pomés, PhD (Indoor Biotechnologies, Inc., Charlottesville, VA, USA)

The location of B cell epitopes in allergens can be useful for the identification of potential allergenic proteins homologous to the allergens. Mapping studies of IgE antibody binding epitopes have traditionally focused, by design, on the identification of linear epitopes, by testing synthetic peptides or recombinant allergen fragments for IgE antibody recognition. However, the three-dimensional (3D) structure of the allergen has been ignored by this strategy, and information on conformational epitopes is lacking. Although conformational epitopes are common in inhaled allergens, food allergens may contain them as well if they are not completely cleaved in the digestive tract, and digestion-resistant fragments are absorbed. From 715 allergens in the WHO/IUIS Allergen Nomenclature database, only ~70 3D-structures of allergens are known, and 21 are from food allergens. In recent years, thirteen structures of seven allergens in complex with antibody fragments have been reported. The antibodies are either IgG that inhibit IgE antibody binding or recombinant IgE from combinatorial libraries. These studies have revealed mechanisms of allergen-antibody interaction and the structure of conformational epitopes, providing insight into molecular basis of cross-reactivity (i.e., Der p 1/Der f 1, see Figure below). Studies like these, that take into account the three-dimensional structure of allergens, are needed to fully understand the B cell repertoire for allergenicity prediction.



**Figure.** X-ray crystal structures of natural Der p 1 and Der f 1 in complex with a fragment of a mAb that inhibits IgE antibody binding (blue and green, respectively). The structures show the molecular basis of cross-reactivity between both dust mite allergens, and a conformational epitope which overlaps with an IgE antibody binding site (Chruszcz M, et al. Molecular determinants for antibody binding on group 1 house dust mite allergens. *J Biol Chem* 2012 Mar 2;287(10):7388-98).



## **Allergen protein families – what makes them so peculiar?**

*Karin Hoffmann-Sommergruber, PhD (Medical University of Vienna, Austria)*

Only a restricted number of protein families account for the majority of food allergic reactions in predisposed individuals. The most important allergenic protein families from plant foods are the prolamin superfamily including the non-specific lipid transfer proteins or the 2S albumins, as well as the cupin superfamily with the 11S and 7S globulins. The panallergen profilin and the Bet v 1-related proteins are connected to pollen-food cross-reactivity. Among the animal food allergens, parvalbumins, tropomyosins, and the caseins followed by the ATP-guanido-phosphotransferases as well as the transferrins are the most important ones. It seems that conserved structures and certain biological activities contribute to the allergenic activity. Within a protein family the presence of highly conserved surface structures and sequence identities above 50% account for clinically relevant cross reactivity. Recently, great efforts have been undertaken to study the physicochemical properties of allergens and to identify relevant IgE-binding epitopes which in turn helped to discriminate between hypo- and hyperallergenic molecules. These well-defined proteins can now be used to study their uptake across mucosal barriers and their interaction with the immune system. Improved knowledge on the specific uptake and processing of allergens will contribute to our understanding about the factors that make a protein an allergen!



## **Implementation of the results from the EU Framework Programme 6 funded project Sens-it-iv**

*Dr. Erwin L. Roggen (Novozymes A/S and 3Rs Management and Consultancy, Bagsvaerd, Denmark)*

The Sens-it-iv project contributed to the 3Rs by expanding existing scientific knowledge, and by applying this knowledge to develop novel tests for identifying skin and respiratory sensitizers. Precision Cut Human Lung Slices (PChLS) are considered to represent the material of choice to correlate ex vivo toxicity in humans with in vitro data. Human lung cell based tests were assessed and subsequently discarded on the basis of the information provided by the PChLS technology. Two 'external' assay systems were identified. The in vitro alveolar-capillary barrier model is to our knowledge the only test to date containing alveolar type I and type II-like cells. A commercially available model employing primary human bronchial epithelial cells (MucilAir™) was found to be a good model for assessing bronchial responses. Both test systems discriminate sensitizers and irritants, as well as respiratory and skin sensitizers. The 'Sens-it-iv' Genomic Allergen Rapid Detection test for the skin builds upon 200 genes found to be affected by skin sensitizers in MUTZ-3 cells. The test was recently shown to identify skin sensitizers with 97% accuracy. Recently a gene signature was identified allowing for the identification of chemical respiratory sensitizers.

These tests are currently being implemented for assessing the hazard of respiratory sensitization related to the use of chemical as well as proteinaceous compounds. For proteins, an integrated approach is under development combining historical, in vitro, and computational data.



## **Translational testing of allergenicity of proteins: from animal testing to *in vitro* testing with mouse intestinal organoids**

Joost Smit<sup>1</sup>, Marianne Bol-Schoenmakers<sup>1</sup>, Rob Bleumink<sup>1</sup>, Jean Paul Ten Klooster<sup>2</sup>,  
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In mouse food allergy models, the mucosal adjuvant cholera toxin (CT) is used to induce innate immune changes that trigger allergen-specific T and B cell responses. These innate immune changes involve activation of intraepithelial lymphocytes (IEL, e.g.  $\gamma\delta$ -T cells), subsets of dendritic cells (DC), and induction of co-stimulatory molecules. We hypothesize that molecular stress imposed on gut epithelial cells, for instance by CT or NSAIDs, is a principal trigger for IEL to subsequently activate DC and T and B cells. To explore this hypothesis, we isolate IEL from mouse intestine and investigate their interaction with a mouse intestinal epithelial cell line (MODE-K) and with stem-cell derived intestinal organoids. The latter consist of epithelial crypts, containing enterocytes, Paneth cells, goblet cells and endocrine cells. Co-culture of MODE-K cells and IEL in the presence of CT results in cytokine secretion of MODE-K, increased degranulation of IEL and upregulation of MHC II on both cell types. Culturing organoids in the presence of IEL-derived supernatant results in increased expression of MHCII and MHCII-related molecules on organoid cells. This *in vitro* co-culture system might contribute to further understanding of early mechanisms of sensitization. Together with specific animal models such *in vitro* models may eventually help to assess allergenicity of new proteins.



## **Tools and technologies for immunogenicity and allergenicity risk management**

*Jeremy Fry, PhD (ProImmune Limited, Oxford, United Kingdom)*

Introduction of novel protein content has enabled major advances in the development of a broad range of consumer products, including foods. However, as these products reach the market, entire populations will be exposed to the newly-engineered proteins they contain. Unwanted immune responses to novel proteins have the potential to cause serious health problems, as new food allergies could develop. Managing the risk of allergenicity in protein development is a complex bioanalytical challenge to address.

This presentation will illustrate, with examples, the range of tools and technologies now available for allergenicity risk management, and in addition discuss when each technique would be best employed. We have developed in vitro methodology to identify neo-epitopes present in engineered proteins and characterize their likely impact (in terms of eliciting a T cell response) in any given population. We are also able to identify the peptide epitopes from novel proteins processed and presented by MHC class II molecules (using mass spectrometry), and have developed methods for comparing whole proteins for their likely allergenicity. Case studies where these technologies have been used to great effect will be presented.

*(See “Resources” tab for more information.)*



## **SESSION IV: HOST-SPECIFIC FACTORS**

### **ABSTRACTS**

#### **The role of the epithelium in sensitization**

*Emily Swindle, PhD (University of Southampton, United Kingdom)*

#### **Dendritic cells: subtypes and how they are activated**

*Maud Plantinga<sup>1,2</sup>, Martin Guilliams<sup>1,2</sup>, Manon Vanheerswynghe<sup>1,2</sup>, Wendy Toussaint<sup>1,2</sup>, Bernard Malissen<sup>4</sup>, Hamida Hammad<sup>1,2</sup>, Bart N. Lambrecht<sup>1,2,3</sup>*

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#### **T-cell subtypes and plasticity: which are relevant in the allergic phenotype?**

*Lars K. Poulsen, PhD, Dr. Med. (Gentofte Hospital, Copenhagen, Denmark)*

#### **B-cell isotype switch and the longevity of the IgE-antibody response**

*Lone Hummelshøj, PhD (Gentofte Hospital, Hellerup, Denmark)*

#### **Sub-populations at risk: age and co-morbidity as risk factors for developing food allergy**

*Jonathan Hourihane, MD, FRCPI (University College Cork, Ireland)*



## **The role of the epithelium in sensitization**

*Emily Swindle, PhD (University of Southampton, United Kingdom)*

The epithelium is a membranous tissue which covers the external surface and lines the internal compartments of organs within the body. At these sites, the role of the epithelium is to form barriers which define boundaries and prevent the unrestricted exchange of materials. Depending on the type of epithelium, it will serve specialized functions relevant to the organ in which it is found. In the conducting airways, the epithelium allows the passage of air to the gas exchange regions of the lung and forms a physical, chemical, and immunological barrier. The physical barrier is composed of a polarised epithelium which is selectively permeable to ions and macromolecules due to the presence of receptors, transporters and tight junctions. The chemical barrier contains secretions including mucus, cytoprotective and host defense molecules which trap and inactivate inhaled particles allowing clearance via the mucociliary escalator. The immunological barrier performs immune surveillance through the expression of innate immune receptors and leads to the activation of effector cells and antigen presenting cells through the release of mediators. This presentation will discuss the components of the physical, chemical and immunological epithelial barrier of the airways and the mechanisms by which proteins may penetrate the epithelium.



## **Dendritic cells: subtypes and how they are activated**

*Maud Plantinga*<sup>1,2</sup>, *Martin Guilliams*<sup>1,2</sup>, *Manon Vanheerswynghe*<sup>1,2</sup>, *Wendy Toussaint*<sup>1,2</sup>, *Bernard Malissen*<sup>4</sup>, *Hamida Hammad*<sup>1,2</sup>, *Bart N. Lambrecht*<sup>1,2,3</sup>

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Lung dendritic cells control T cell polarization towards a Th1, Th2 or Th17 response, or conversely, prevent harmful immune responses to inhaled antigen via regulatory T cells. In the steady-state lungs, two subsets of conventional DCs (cDCs) have been identified: CD103<sup>+</sup> cDCs and CD11b<sup>+</sup> cDCs. Importantly, equivalence of mouse DCs with respectively the BDCA-3<sup>+</sup> and the BDCA-1<sup>+</sup> human DC subsets has been proposed. In addition to the conventional DCs, we have recently reported that during HDM sensitization a third subset of so-called inflammatory DCs can be found in the lungs. These inflammatory DCs express FcεRI during HDM exposure, which suggests that expression of a high affinity Ig receptor may be useful to identify monocyte-derived inflammatory DCs in the lungs. Moreover, as the identity of the major DC subset responsible for Th<sub>2</sub> induction during HDM-specific allergic airway sensitization remains unknown, we set out to unravel the role of CD103<sup>+</sup> cDCs, CD11b<sup>+</sup> cDCs, and inflammatory DCs in a mouse model for HDM-specific sensitization. This is important, because one of the most common allergens to which asthma patients are sensitized is the house dust mite (HDM). Understanding the role of distinct lung DC subsets might lead to the discovery of specialized therapies.



## **T-cell subtypes and plasticity: which are relevant in the allergic phenotype?**

*Lars K. Poulsen, PhD, Dr. Med. (Gentofte Hospital, Copenhagen, Denmark)*

The allergic immune response has previously been characterized by IgE, eosinophilia, and a T-cell response, including the cytokines IL-4, IL-5, and IL-13. More recently, cytokines such as IL-9, IL-22, and IL-17 have also been incriminated as participating in the allergic phenotype with CD4+ T-cell subsets being defined by those very cytokines: Th9, Th22, and Th17. It is still debatable, however, whether all subtypes represent unique differential paths or whether several "cassettes" of stimulus-response-cytokine expression may co-exist in the same CD4+ T-cell. One such "cassette" is seen with the IL-1 family of cytokines (IL-1beta, IL-18, and IL-33) which may modulate the expression of the cytokines such as IL-9 and IL-10 by other T-cell subsets. Other such modulatory cytokines are TSLP and IL-25 as well as some of the factors leading to the formation of the follicular T-helper cells that are believed to be important for the B-cell differentiation.



## **B-cell isotype switch and the longevity of the IgE-antibody response**

*Lone Hummelshøj, PhD (Gentofte Hospital, Hellerup, Denmark)*

A key event in the pathogenesis of allergy is the production of IgE antibodies. In order for a B cell to switch to IgE production, it needs two signals provided by a Th2 cell. The first signal is delivered by the cytokines IL-4 or IL-13, which target the C $\epsilon$  gene for initiating switch recombination. The second signal is delivered by interaction of the cell surface antigen CD40 with its ligand (CD40L) expressed on activated T cells. Once the IgE positive B cells are formed, they are able to differentiate into non-dividing, IgE-producing plasma cells. Although some populations of long-lived plasma cells persist in the spleen, most of them return to the bone marrow or invade inflamed tissues, where they survive up to several months or even lifetime in survival niches as resident, immobile cells. Long-lived IgE-producing plasma cells are difficult to target therapeutically, thus emphasizing the need for more knowledge on preventable causes of IgE and allergy development.



## **Sub-populations at risk: age and co-morbidity as risk factors for developing food allergy**

*Jonathan Hourihane, MD, FRCPI (University College Cork, Ireland)*

Food allergy is an immune mediated adverse response to foods, usually to proteins. Unsurprisingly food allergy is most prevalent in infants and young children, which is the natural and inevitable window of first encounters with foreign antigens, including food antigens. The integrity of the gastrointestinal mucosal immune defenses and altered skin barrier function have both been implicated in deviant/allergic responses to foods. Circulating T lymphocytes in food allergic children show patterns of cell membrane markers related to skin, whereas food tolerant children show GI homing patterns, implying failure of oral tolerance may be due to food allergen encounter through the non-tolerogenic skin route. It is for this reason that a major current focus of food allergy research is into firstly examining early introduction of allergenic foods by mouth to promote tolerance, and secondly examining the role of the skin barrier in sensitisation. Geographic and inter-cultural differences are also apparent. The introduction of new foods into a population's diet affects adults in ways that suggest they have become sensitised via inhalant plant allergens such as pollens, rather than directly by the implicated food.



## **SESSION V: ANIMAL MODELS FOR SENSITIZATION**

### **ABSTRACTS**

#### **The BALB/c mouse model of allergy for the assessment of sensitizing properties of proteins and foods and their alteration by environmental conditions**

*Dr. Jean-Michel Wal (INRA-CEA SACLAY, France)*

#### **The rat brown Norway model to assess the oral sensitizing properties of food proteins**

*André H. Penninks, PhD, ERT (TNO Triskelion BV, Zeist, The Netherlands)*

#### **Limitations and possibilities of animal models for human allergenic risk evaluation**

*Charlotte B. Madsen, Stine Kroghsbo, Katrine L. Bøgh (Technical University of Denmark, Søborg, Denmark)*

#### **The mouse cholera toxin model for evaluating protein allergenicity**

*Joost Smit, Raymond Pieters (Institute for Risk Assessment Sciences [IRAS], Immunotoxicology, Utrecht University, The Netherlands. [IRAS is a partner in the Utrecht Centre for Food Allergy.]*

#### **Conclusions: How close are we to predicting allergenicity of new proteins?**

*Corinne Herouet-Guicheney, PhD (Bayer SAS, Bayer CropScience, Sophia Antipolis, France)*



## **The BALB/c mouse model of allergy for the assessment of sensitizing properties of proteins and foods and their alteration by environmental conditions**

*Dr. Jean-Michel Wal (INRA-CEA SACLAY, France)*

The assessment of the allergy risk of a novel protein mainly focuses on its potential to elicit an allergic reaction in consumers already sensitized to a cross reacting protein. However, reliable tests are missing to predict its potential to sensitize *de novo* atopic individuals which actually depends not only on intrinsic properties of the protein but also on complex interactions with the genetic background and physiology of the consumers and with environmental conditions.

In this regard, integrated animal models such as those developed with the BALB/c mouse, a Th2 biased responder strain, may provide useful information. Sensitization to purified proteins or whole foods is achieved using experimental conditions and is qualitatively and quantitatively assessed in terms of intensity and specificity of the response. Currently, such models are not intended to predict the risk of predisposed humans to develop allergy in everyday life conditions but rather to compare the sensitizing properties of different allergenic proteins in varying conditions. Moreover, they allow assessing the alteration of the sensitizing potential in relation to changes regarding the modes of exposure or the environmental factors. Such an approach will help to better understand the mechanisms that favour the induction of allergic reaction vs oral tolerance.



## **The rat brown Norway model to assess the oral sensitizing properties of food proteins**

*André H. Penninks, PhD, ERT (TNO Triskelion BV, Zeist, The Netherlands)*

From the first ILSI/IFBiC decision tree approach proposed in 1996, which was entitled “Assessment of the Allergenic Potential of Foods Derived from Genetically Modified Crop Plants,” it was clear that there is a great need for accepted animal models to study the sensitizing potential of new food proteins. However, at that time, it was already evident that it would not be easy to meet the most important criteria in one model. Selection of species and strain, route of exposure for sensitization and challenge, as well as adjuvant use, were important criteria to consider. We selected the Brown Norway (BN) strain of rats, a high immunoglobulin (particularly IgE) responder rat strain, to evaluate the intrinsic potential of food proteins to induce an allergic sensitization by gavage dosing in the absence of an adjuvant. In these studies BN rats were exposed to whole foods (cow’s milk), total protein extracts (hen’s egg-white, peanut) or purified strong or weak allergenic proteins [e.g., ovalbumin(OVA), Ara h1 from peanut, tropomyosin from shrimp (Pen a1) and beef, patatin (sol t1) from potatoes and 2S albumine] upon daily gavage dosing for 42 days without the use of an adjuvant. In studies with soy, it was also demonstrated that unintended dietary pre-exposure of test animals and their parental generation to the protein source to be evaluated may critically affect the oral sensitizing potential of the test protein(s).

In the presentation, the results of our studies in the BN rat allergy model will be summarized and, together with results published by others, the possibilities and limitations of the BN rat model will be discussed.



## **Limitations and possibilities of animal models for human allergenic risk evaluation**

*Charlotte B. Madsen, Stine Kroghsbo, Katrine L. Bøgh (Technical University of Denmark, Søborg, Denmark)*

There are many unanswered questions relating to food allergy sensitization in humans. We don't know under what circumstances sensitization takes place, i.e., route (oral, dermal, respiratory), age, dose, frequency of exposure, infection, or by-stander effects of other allergens. In addition, we don't know under what circumstances oral tolerance develops. With all these unanswered questions, it is a big challenge to design an animal model that, with relatively few animals, is able to predict if a food allergen is not only a potential allergen but also will predict its potency, a prerequisite for risk evaluation. One of the pitfalls may be the premise that an animal model needs to mimic the disease. Chemical contact sensitizers may be predicted in an animal test, i.e., the Local Lymph Node Assay (LLNA). This assay is based on detailed mechanistic knowledge of contact sensitization including knowledge on dose-response relationship. The outcome of the test is sensitization measured as cell proliferation in the regional lymph node.

Animal models in food allergy can be used to increase our understanding of food allergens and food allergy sensitization, e.g., the influence of digestion or processing or to compare closely related allergens. Examples of this will be given.



### **The mouse cholera toxin model for evaluating protein allergenicity**

*Joost Smit, Raymond Pieters (Institute for Risk Assessment Sciences [IRAS], Immunotoxicology, Utrecht University, The Netherlands. [IRAS is a partner in the Utrecht Centre for Food Allergy.]*

Without doubt, animal models have contributed to insight into the mechanisms of sensitization to food proteins and development of food allergy. In mouse models, the mucosal adjuvant cholera toxin (CT) is generally used to induce allergic sensitization to co-administered proteins in mice, while feeding the protein alone induces oral tolerance. Although the principal allergic response in this model is driven by CT, the properties of the administered protein may be even more decisive. In detailed studies in this mouse model, we investigated mechanisms of anaphylaxis and the relative contribution of the individual peanut allergens to clinical food allergic responses. Individual peanut allergens differed significantly in their capacity to sensitize mice. Interestingly, depending on the route of provocation, peanut proteins differed even more in their capacity to cause allergic effector responses such as mast cell degranulation and systemic anaphylaxis. In further studies, the mechanism behind these functional differences of individual peanut allergens and their cross-reactivity on T cell and antibody level was investigated. These findings are relevant to risk assessment of novel proteins and illustrate the usefulness of mouse food allergy models to examine sensitization and effector responses to potential allergens at different levels in the allergic cascade.



## **Conclusions: How close are we to predicting allergenicity of new proteins?**

*Corinne Herouet-Guicheney, PhD (Bayer SAS, Bayer CropScience, Sophia Antipolis, France)*

A number of foods containing genetically modified (GM) crops have been introduced to the marketplace. These products have been carefully evaluated for their overall safety from an agronomic, environmental, performance, and equivalence perspective. Furthermore, several studies have confirmed the safety of the GM crops and the newly expressed protein(s) associated with the new trait(s).

One aspect of the protein safety evaluation is the question concerning their potential allergenicity. The allergenicity risk to consumers from GM crops may be placed into one of three categories. The first risk category involves the transfer of a known allergen or cross-reacting allergen into a food crop. The second risk category is the potential for increasing the allergenicity of a crop by increasing the expression of endogenous allergens. The third risk category involves expression of proteins that may become allergens *de novo*.

Approaches to identifying potential risks have been developed and modified over the past 15 years. However, no single factor has been recognized as the primary indicator for the allergenic potential of proteins, and no validated animal model that is predictive of protein allergenicity is currently available. Therefore, the evaluation of protein allergenicity is currently based upon a 'weight of evidence' approach, which takes into account a variety of factors that have been associated with allergens, such as stability to pepsin digestion or other enzymes *in vitro*, glycosylation status, protein abundance in the crop, homology to known allergens, and the safety of the source if the gene(s). In addition, as part of the 'weight-of-evidence' assessment, the GM crop endogenous allergen levels are compared to those of its non-GM comparator to make sure that the genetic modification does not impact known endogenous allergens naturally present in the host crop.

This presentation provides an overview of the current 'state of the art' for evaluating potential allergenicity of proteins expressed in GM crops.