

Food Allergy: a global perspective

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX: October 23rd, 2009

Food allergy has now reached epidemic proportions in the UK where the personal, social and economic cost extends not only across the nation but affects other developed countries as well. This has prompted the House of Lords Science and Technology Committee to publish its report on allergy (September 2007) in which they conclude that evidence-based research into food allergy is urgently needed to ensure that sound scientific evidence underpins public policies.

The aim of this first EuroScicon meeting on Food Allergy is to provide a wider perspective, being unlimited in scope. It strives to provide a broad overview of the following;

- food allergies and intolerances
- the susceptibility to food allergy
- early life origins of food allergy
- food tolerance versus food sensitisation
- effects of food processing on food allergenicity
- industrial dimensions of food allergy
- food allergy and the processing of protein allergens by antigen-presenting cells
- novel foods

Meeting chairs; *Dr. Kamal Ivory*, Institute of Food Research, Norwich, UK and *Professor Claudio Nicoletti*, Institute of Food Research, Norwich, UK

This meeting has CPD accreditation

9:00 – 9:45 Registration

9:45 – 10:00 Morning Session - Introduction by the Chair: *Professor Claudio Nicoletti*, Institute of Food Research, Norwich, UK

10:00 – 10:30 Primary prevention of food allergy - do pre or probiotics have a role?

Dr Robert J Boyle, Imperial College London, UK

The health benefits of probiotic bacteria or prebiotic oligosaccharides are controversial. In this talk Dr Boyle will describe the scientific rationale for using these interventions to prevent allergic disease, and discuss recent insights into their biological and clinical effects.

10:30 – 11:00 Oral tolerance versus food allergic sensitization

Professor Stephan Strobel, Director of Postgraduate Clinical Education and Chair of the Peninsula Postgraduate Health Institute The Peninsula Medical School

Clinical non-responsiveness to food antigens (Oral Tolerance) is the mucosal default mechanism in about 95% of the population. Timing of antigen (food) administration during the peri- and postnatal period are important, although their effects on the development of tolerance or food allergies are still not entirely understood. The primary mechanisms by which tolerance may be mediated indicate a central immunoregulatory of T lymphocytes which secrete Transforming Growth Factor beta and Interleukin 10. Additional mechanisms include T cell deletion, anergy, suppression, and apoptosis. This presentation will summarise our understanding of the underlying mucosal immunoregulatory events. The role of innate immunity and the effects of probiotics and specific tolerance induction will be assessed.

11:00- 11:10 Speakers photo

11: 10 – 11:30 Mid-morning break and poster viewing

11:30 – 12:00 Factors affecting the development of food allergy

Professor Ian Kimber, University of Manchester, UK

This presentation will explore the factors that influence the acquisition of allergic sensitisation to dietary proteins. Among those that will be considered are: the factors that confer on proteins the ability for induce allergic responses, the basis for inter-individual differences in susceptibility to food allergy, and the impact that exposure (route, timing and duration of exposure) has on the development of sensitisation.

- 12:00 – 12:30 **New treatment options in food allergy**
Professor Jonathan Hourihane, University College Cork, Ireland
- 12:30 – 13:45 **Lunch and Poster Viewing**
- 13:45 – 14:00 **Afternoon Session - Introduction by the Chair:** *Professor Claudio Nicoletti, Institute of Food Research, Norwich, UK*
- 14:00 – 14:30 **Oleosins; new and important allergens in legumes, nuts and seeds?**
Dr Laura Zuidmeer, Academic medical Center, Amsterdam
Patients clinically reactive to peanut, tree nuts and/or seeds can score negative in various diagnostic tests. Oleosins are oilbody-associated proteins that have been tentatively implicated in allergic reactions to these foods. We isolated natural oleosins from various sources, determined their allergenicity to nut and seed allergic patients and defined their presence in commercially available extracts. We conclude oleosins might play a role as highly prevalent allergens which can evoke systemic reactions, as association with oilbodies may protect from rapid proteolysis after ingestion. Due to extensive defatting of extracts used for *in vitro* diagnostics, oleosin-specific IgE can easily be missed.
- 14:30 – 15:00 **Industrial dimensions of food allergy.**
René Crevel, Unilever Safety and Environmental Assurance Centre, UK
Food allergy is now recognised as a significant public health issue, which needs to be managed. Much experience has now been gained with such systems and the general principles that govern good practice are reasonably well understood. This presentation will briefly review the principles underlying allergen management, consider gaps in knowledge that limit the ability to assess the risk from allergens accurately and attempt to draw conclusions about the adequacy of current systems.
- 15:00 – 15:30 **Afternoon Tea/Coffee and Last Poster Viewing**
- 15:30 – 16:00 **The allergenic potential of novel foods**
Dr. Jean-Michel WAL, Directeur du Laboratoire d'Immuno-Allergie Alimentaire, France
The pre-market approval of Novel Foods requires the assessment of their allergenicity, i.e. the capacity to trigger an allergic reaction in individuals already sensitized to cross reactive proteins and/or to de novo sensitize predisposed individuals. In the case of genetically modified organisms (GMOs) this includes the allergenicity of both the newly expressed (trait) proteins and the whole GM food. The presentation will analyse what the allergy risk of Novel Foods actually is and give an overview of the latest developments in allergenicity testing of GMOs and on the strategy recommended by scientific Committees and particularly EFSA for the assessment of their allergenicity.
- 16:00– 16:30 **Digestibility, allergenicity and the safety of novel proteins**
Emily Foster, University of Manchester
Resistance to digestion is used as one of the criterion for the safety assessment of novel proteins. A more detailed appreciation of the relationship between digestibility and allergenicity is required. Here the hypothesis that protein allergenicity relates more closely with stability to digestion within antigen presenting cells (APC) than it does with stability to digestion with pepsin will be discussed.
- 16:30 – 17:00 **Chairman's summing up**

*This meeting was **organised by Euroscicon** (www.euroscicon.com), a team of dedicated professionals working for the continuous improvement of technical knowledge transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry. The event was hosted by **'BioPark** (www.biopark.co.uk), a research and development centre in Welwyn Garden City providing specialist facilities and support for bioscience and health technology businesses to grow, and to develop new products and technologies*

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About the Chairs

Prof. Claudio Nicoletti. After a degree in Biological Sciences at the University of Siena (Italy) Claudio Nicoletti spent the early part of his career in the United States, initially at the Dept. of Microbiology and Immunology of the University of Texas at Galveston and then at the Dept. of Immunology of the University of Maryland at Baltimore investigating cellular and molecular aspects of immunosenescence. Then after two years at the German Cancer Centre at Heidelberg as a post-doctoral fellow he joined The Babraham Institute in Cambridge to work on several aspects of gastrointestinal immunity. At the Institute of Food Research his role is to lead research in the field of gut immunology with emphasis on the interactions between food-borne pathogens and the intestinal immune system and on regulation of immune responses in allergy.

Dr Kamal Ivory is a project scientist in the Mucosal Immunology group at the Institute of Food Research in Norwich. She began her research career looking at ways to prevent kidney allograft rejection at Guy's Hospital in London. From there Kamal moved to the Royal Free Hospital (RFH) where she obtained her PhD. She continued to work there on ways of exploiting the potential of monoclonal antibodies for diagnosis and therapy, while also participating in ageing and immunodeficiency research. On moving to the Institute of Food Research in Norwich Kamal initially worked on healthy ageing, but is currently studying immune regulation in food allergy

About the Speakers

Professor Ian Kimber, is currently Professor and Chair of Toxicology at the University of Manchester. Previous to that he was Head of Research and Principal Fellow at the Syngenta Central Toxicology Laboratory. He has broad research interests based around immunotoxicology, allergy and skin biology with specific research themes currently including: the pathogenesis of food allergy, the stimulation of T lymphocyte responses by skin sensitising chemicals and respiratory allergens, and the molecular regulation of Langerhans cell function and the roles played by these cells in the orchestration of cutaneous immune responses. In addition, Professor Kimber has active interests in the development, validation and application of novel predictive test methods in toxicology, and in research that seeks to reduce, refine and replace the use of animals in safety assessment. Professor Kimber holds, and has held, a wide variety of positions on national and international expert and scientific advisory committees. Currently these include the following: UK Medical Research Council (MRC) Training and Career Development Board, Special Advisor to the MRC on Industrial Liaison, UK Medicine and Health Regulatory Agency (MHRA) Committee for Safety of Devices, Programme Advisor Food Standards Agency Food Allergy and Intolerance Research Programme, and member OECD Expert Committee on Sensitisation. He has published over 500 research papers, review articles and book chapters and serves currently on the editorial boards of toxicology, immunology, dermatology and pathology journals. Professor Kimber has received a number of awards and prizes. These include: the SmithKline Beecham Laboratory Animal Welfare Prize (2000), the 9th Robert A Scala Award in Toxicology, the Doerenkamp-Zbinden Foundation Prize for Realistic Animal Protection in Biomedical Research (2001), Society of Toxicology Enhancement of Animal Welfare Award (2003), and Society of Toxicology Immunotoxicology Career Achievement Award (2005).

Professor John O Warner, Qualified in Sheffield 1968, paediatric training in Great Ormond St, Hosp., consultant at Brompton Hospital from 1980-90, Professor of Child Health Southampton 1990-2006, Professor of Paediatrics and Head of Dept. Imperial College, London from 2006. Editor in Chief Pediatric Allergy and Immunology. Research focus on the early life origins of asthma and related allergic disorders. Published over 300 papers on this topic

Dr Laurian Zuidmeer finished her PhD in molecular plant virology at Leiden University, then moved to the Allergy group headed by Dr. Ronald van Ree in Amsterdam. Here she worked in two subsequent European consortia (SAFE and EuroPrevall) on the prevalence, cost and basis of food allergy. Her main topics were the isolation and molecular characterisation of plant food allergens (in particular fruits, legumes, nuts and seeds) and determining their prevalence in food allergic patient groups. Part of her work also involved setting up an assay to measure differences in avidity/affinity of IgE antibodies.

Emily Foster has a degree in Biomedical Science from the University of Manchester. As part of her degree she spent one year in Germany working at Boehringer Ingelheim on lung pharmacology characterising murine models for asthma. After her degree her interest in immunology and allergy continued and she is now in the second year of her PhD at the University of Manchester.

René Crevel works as a Science Leader at Unilever's Safety and Environmental Assurance Centre. His principal responsibilities include providing scientific advice and guidance on possible adverse effects of materials and their use, arising from their interaction with, or modulated through the immune system. In particular, he is responsible for advice and guidance on food allergy and allergen management to Unilever Companies, and for leading Unilever's food allergy research programme. He has published papers and book chapters on various aspects of food allergy, including determination of the allergenicity of novel proteins, post-launch monitoring and risk assessment and management of food allergens.

Professor Stephan Strobel studied for his PhD in Mucosal Immunology with the late Professor Anne Ferguson at Edinburgh University, Scotland. He was appointed New Blood Lecturer at the Institute of Child Health and Consultant in Immunology at Great Ormond Street Hospital in 1985. He has published extensively relating to immunodeficiency, gastrointestinal immunoregulation and allergic sensitisation. In 2004 he was appointed Founding Director of the Peninsula Postgraduate Health Institute of the Peninsula Medical School. He has received several international prizes for his scientific work and is currently Chair of working groups and expert adviser to the Nutrition, Dietetics and Allergy Panel of the European Food Safety Authority (EFSA).

Professor Jonathan Hourihane is Professor of Paediatrics and Child Health in University College Cork, Ireland. He graduated from Trinity College Dublin in 1987 and entered paediatric training in the UK in 1990. He completed his specialist training in Allergy, Immunology and Infectious Diseases at the Institute of Child Health and Great Ormond Street Hospital in 2000. He is a Board member of the Paediatric section of EAACI and was the founding chairman of the Paediatric Group of BSACI. He is an Associate Editor of the journals *Clinical and Experimental Allergy* and *Paediatric Allergy and Immunology*. He is a member of the Research Advisory Board of the US based Food Allergy and Anaphylaxis Network, and is a medical adviser to the Anaphylaxis Campaign in the UK

Dr Robert Boyle trained as a Paediatric Allergist at the Royal Children's Hospital, Melbourne. He studied the mechanisms by which probiotic bacteria may prevent allergic disease for his PhD thesis, and is involved with ongoing clinical studies of prebiotics and food allergy pathogenesis in his current role as Lecturer in Paediatric Allergy at Imperial College London.

Dr. Jean-Michel Wal is director of the Food Allergy Laboratory of INRA. He conducts research programmes on the structure-allergenicity relationship and develops new models for the assessment and prevention of the food allergy risk. Dr. Wal is a member of scientific societies for nutrition, immunology and allergology and of several scientific and advisory committees in charge of the safety risk assessment of (Novel) Foods. He is currently a member of the GMO Panel of the European Food Safety Authority (EFSA) in which he chairs the Working Group on Assessment of Allergenicity of genetically modified organisms.

POSTER PRESENTATIONS

ASSESSMENT OF PROTEIN ALLERGENICITY IN MICE : RELATIONSHIP TO IMMUNOGENICITY

R.J. Dearman, I. Kimber

University of Manchester, Michael Smith Building, Oxford Road, Manchester, M13 9PT

With the increasing interest in the development of novel foods derived from transgenic crop plants there is a growing need for approaches to the characterisation of the allergenic potential of proteins. Whereas immunogenicity is an almost universal property of foreign proteins, including food proteins, relatively few are significant dietary allergens with the inherent capacity to provoke IgE antibody production and immediate type hypersensitivity responses.

In the current experiments, the ability of a range of proteins to induce specific IgG and IgE antibody responses following systemic (intraperitoneal) exposure to BALB/c strain mice has been examined. In this context, IgG antibody is a marker of immunogenicity and IgE antibody production is indicative of allergenic potential. Antibody responses have been measured in the absence of adjuvants such as cholera toxin, in order to characterise the intrinsic ability of the proteins to provoke immune and allergic responses. Specific IgG antibody has been measured by enzyme-linked immunosorbent assay (ELISA) and specific IgE antibody has been measured by homologous passive cutaneous anaphylaxis (PCA) assay.

Intraperitoneal administration of proteins not generally associated with allergic responses such as potato lectin and purified potato protein stimulated vigorous IgG antibody responses but failed to stimulate IgE antibody production, despite testing at relatively high doses (10%). Exposure of BALB/c strain mice to protein enzymes such as lipolase and termamyl that cause IgE-mediated respiratory allergy induced relatively low titre IgG antibody responses, but comparatively vigorous IgE antibody production. Similarly, intraperitoneal administration of the major cows' milk allergen β -lactoglobulin and the peanut allergen Ara h 1 stimulated weak IgG antibody responses and detectable specific IgE antibody production. In contrast, exposure to the peanut allergen Ara h 2 failed to provoke detectable IgG or IgE antibody. The relatively poor immunogenicity of both of the peanut proteins may reflect prior exposure to cross-reactive soy proteins in the diet.

These data demonstrate the importance of monitoring IgG antibody responses, such that only the failure to observe detectable IgE antibody in the presence of a robust IgG antibody response is interpreted as a secure negative. Furthermore, respiratory sensitizing proteins may also be characterized as a function of induced IgE antibody responses following systemic intraperitoneal exposure. Experience to date is encouraging that this method may represent a useful approach for the prospective identification of protein allergens.

CROSS TALK BETWEEN LYMPHOCYTES AND INTESTINAL EPITHELIAL CELLS IN THE GUT: AN IN VITRO CO-CULTURE MODEL

A.L. Man, M.S. Winterbone and C. Nicoletti

Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, E. Anglia

The mucosal epithelial layer lining the gastrointestinal tract fulfils a dual function. Firstly, it forms a protective barrier which prevents the adherence of macromolecules and bacteria, present in the intestinal lumen, to the host epithelial cells and this prevents their entry into the cell. Secondly, certain areas of the epithelium, the Peyer's patches, represent major sites of antigen and micro-organism sampling. The mucosa-associated lymphoid tissues (MALT), underlying the follicle associated epithelium (FAE), are derived from the closely associated crypts and migrate upwards towards the apical region of the dome. Lymphoid follicles are embedded within the villi. Antigenic molecules and bacteria are taken up by cells of the FAE, presented to lymphocytes residing in the lymphoid tissue of the MALT, giving rise to an immune response. Microfold or membranous (M) cells are the specialised epithelial cells of the FAE which carry out a 'gatekeeper' role and are involved in the sampling of antigens. They continuously take up and internalise contents from the lumen and transport this material to the underlying organised lymphoid tissue.

We have devised an *in vitro* transwell model, comprising of a cluster plate and an insert well, to study FAE. On the base of the insert well is a polycarbonate membrane and cells from a human colon carcinoma cell line (Caco2) can be seeded onto this membrane. Tissue culture medium can be added to the lower and upper compartments of the transwell. Caco2 cells can then be grown to confluence on the membrane. The cells are grown in a fixed orientation and this provides a useful *in vitro* model for the gut. The upper and lower chambers represent the gut lumen and the basolateral side of the cell, respectively. Furthermore, it has been shown that co-culture of the monolayer with lymphoid cells derived from Peyer's patch and human B lymphoid (Raji) cell suspension culture will induce a conversion of the Caco2 enterocytes to M-like cells. We are interested in using this system as an *in vitro* model for the FAE and the M cell in experiments to study the function and development of the M cell, and the interaction of these cell types with food-borne pathogens.

One approach to identify the 'factor' that may be involved in the conversion of enterocytes to M cells is the use of microarray technology, to provide information about the expression of genes induced or repressed by the treatment of Caco2 cells with lymphoid cell cultures or their supernatants. Using such an approach in our experimental systems with Jurkat E6 whole cell suspension treatment, we found that 40 genes showed a 2-fold increase in their expression and 26 genes were upregulated after treatment with Raji B whole cell suspension culture. Generally, fewer genes appeared to be affected by treatments with supernatants from Raji B and Jurkat T cell cultures. These cDNA are currently being sequenced to ascertain their identity.

The manner in which M cells interact with different strains of bacterial is also of interest. Bacteria taken up by M cells, via the process of phagocytosis, eventually leads to the presentation of bacterial antigens to lymphocytes for the elicitation of a host immune response. It has been suggested that bacterial strains interact differently with M cells. This is dependent on factors such as invasive ability and virulence. Such factors include resistance to M cell internalisation (*E. Coll*), transportation of the bacteria via the M cell to macrophages in the sub-mucosa (*Campylobacter*) and transcytosis resulting in bacterial invasion which leads to an acute inflammatory response (*Shigella*). Bacteria can be added to the upper transwell chamber onto Caco2 cells that have undergone an M cell conversion. In a preliminary bacterial translocation experiment we found that the number of bacteria that had translocated across the layer, permeability of the monolayer and the effects on M cell morphology could all be investigated. In the same experiment we found 100% translocation/invasion of the cell layers by *Salmonella* wild-type strain SL1344 while a second *Campylobacter* (11168) strain did not cross the monolayer after a 4-hour time period. An increase in translocation of CD31 was seen in monolayers treated with supernatants derived from Raji B and Jurkat E6 T cell suspension cultures, when compared to untreated cells.

Our future aims are to sequence cDNAs, identified by microarray analysis, shown to be affected by treatment with Raji B and Jurkat E6 T cell suspension cultures or their supernatants, and to analyse further any particular genes of interest. We also aim to optimise a fluorescent latex bead assay so that it may be used to measure M cell function. We also aim to further investigate M cell-enteropathogen interactions using the transwell culture system.

DIGESTIBILITY, ALLERGENICITY AND THE SAFETY OF NOVEL PROTEINS

ES Foster, I Kimber, RJ Dearman,

Michael Smith Building, Manchester University, Oxford Road, Manchester, M13 9PT

One criterion used for assessment of the allergenic potential of novel dietary proteins, is resistance to pepsin digestion in a simulated gastric fluid (SGF); the interpretation being that allergenic proteins display higher levels of stability. However, the relationship between resistance to pepsin and protein allergenicity is not absolute, with examples of stable non-allergens and labile allergens reported. The hypothesis being investigated currently is that protein allergenicity relates more closely with stability to digestion within antigen presenting cells (APC) than it does with stability to digestion with pepsin.

Dendritic cells (DC) are professional APC that are essential for the induction of primary immune and allergic responses. Murine bone marrow-derived dendritic cells (BM-DC) precursor cells were isolated from the tibia and fibia of female BALB/c strain mice (6-8 weeks old). BM-DC were cultured for 4, 6 or 8 days in the presence of granulocyte/macrophage colony-stimulating factor. DC phenotype was characterised by membrane marker expression measured by flow cytometry. Expression of MHC class II (Ia), the co-stimulatory molecule CD86, the adhesion molecule intercellular adhesion molecule-1 (ICAM-1; CD54) and the DC-specific marker CD11c (α_2 -integrin) was investigated. Cell surface markers Ia, CD11c and CD86 were expressed constitutively on approximately 20-30% of BM-DC on day 4 of culture. By day 8 of culture the percentage positive cells had increased such that more than 80% of cells expressed Ia and approximately 70% of cells were positive for CD11c and CD86. The profile for CD54 was different in that approximately 90% of cells expressed this marker on day of 4 of culture and similar numbers were observed after 6 and 8 days of culture.

Cathepsins are a family of enzymes found in APC known to be involved in antigen degradation and processing. It has been reported previously that certain cathepsins such as cathepsin D and E are expressed by DC. The kinetics of expression of cathepsin D within BM-DC was characterised by intracellular staining using flow cytometry. Cells were stained for surface markers Ia and CD11c prior to fixing the cells with paraformaldehyde and permeabilising with saponin. Intracellular expression of cathepsin D was detected using biotinylated antibody followed by secondary antibody. Cathepsin D was not detectable in immature day 4 or day 6 DC which were positive for cell surface markers Ia and CD11c. However, preliminary data suggests that on day 8 of culture approximately 80% of Ia⁺ CD11c⁺ cells were positive for cathepsin D.

These data demonstrate that as DC mature in culture, increasing levels of antigen-processing enzyme cathepsin D is detected. In subsequent experiments, the ability of DC subcellular fractions and recombinant cathepsin D to digest allergenic and non-allergenic proteins will be examined.

IMMUNOLOGY OF FOOD ALLERGY – ALTERATIONS OF MULTIPLE IMMUNOREGULATORY PATHWAYS IS REQUIRED TO INDUCE ALLERGIC REACTIONS

J.N, Temblay, E. Bertelli, M. Regoli and C. Nicoletti

Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, E. Anglia

The prevalence of common allergic diseases has trebled in the last twenty years, resulting in approximately one-fifth of the UK population likely to be seeking treatment for allergy. In particular, food allergy has become such a major health concern in Europe over the past two decades that it has been ranked as the fourth world scourge by the World Health Organisation. Immunoglobulin E (IgE)-mediated allergic reactions to food components are serious and life-threatening conditions and because of our limited understanding of their cellular and molecular basis a therapy is not available yet, strict avoidance of the causal food is the only safe approach.

We undertook our studies in order to dissect in detail some aspects of the regulation of immune responses in allergy, such as the production of regulatory molecules (lymphokines) and an intimate interplay between two cell types, dendritic cells and T cells that play a central role in orchestrating finely balanced immune responses. Mice of different genetic backgrounds and with different susceptibility to food allergy were employed in our study. Our idea was to identify specific molecules and signals that were altered or missing in allergy-susceptible mice but not in allergy-resistant mice. We reasoned that by comparing the immunological make-up of these mice we could identify specific pathways that could serve as therapeutic targets for food allergy.

We found that the C3H/HeJ mouse strain showed a much greater susceptibility to food allergy than the Balb/c mouse strain. The latter did not undergo a type 1 allergic reaction after administration of peanuts combined with an adjuvant. Indeed, while 83% of C3H/J mice displayed symptoms of strong anaphylactic response, none of the Balb/c mice did so despite the presence of significant serum levels of peanut-specific IgE antibody in both mouse strains. This demonstrates that C3H/HeJ mice are susceptible to food allergy while Balb/c mice are not.

Next, we extended our investigation to regulatory events within the immune systems of these two mouse strains. We found the existence of interplay between dendritic (DCs) and T cells. DCs play a central role in shaping the magnitude and quality of immune responses. They act as antigen-presenting cells and as such DCs internalize antigens and present them to T cells to initiate appropriate immune responses. DC-T cell cross talk must be strictly regulated and after this interaction T cells kill the DCs in order to control an otherwise uncontrollable immune response. We have observed that in both allergy-susceptible and allergy-resistant mice the interplay between DC and T cells is altered. Indeed, a large proportion of DCs tend to survive T cell-mediated killing mechanisms. However, the observation that this regulatory mechanism is altered in both allergy susceptible and resistant mouse strains strongly suggests that the alteration of this mechanism is probably linked to the production of peanut-specific IgE antibody but it is not enough to induce the development of a food allergy reaction.

Lymphokines are known as soluble mediators of immunity. These proteins are released by cells of the immune system and they deliver activation and/or suppression signals to other target cells of the immune system. A well-balanced production of these molecules is central to the generation of finely tuned immune responses. Here we report that the production of IL-12, a molecule that exerts a very important role in balancing immune responses is severely impaired in the gut immune system of allergy susceptible mice. In contrast, we have observed that DCs from allergy-susceptible mice stopped producing IL-12 when challenged with a T cell-derived stimulus (CD40) and cultured in the presence of another regulatory lymphokine, IL-4, but not in the presence of others, such as IFN- γ .

These data show that the development of food allergy is linked to the simultaneous failure of different regulatory mechanisms. Furthermore, we have identified that the intestinal immune system does not produce appropriate levels of IL-12 in allergy-susceptible mice. We hypothesize that restoring levels of IL-12 in the gut might be a novel and effective strategy for the treatment for food allergy.

THE EFFECT OF GLYCOSYLATION ON THE INDUCTION OF IgE ANTIBODY.

R.J. Almond^{1,2}, B.F. Flanagan¹, I. Kimber², R.J. Dearman²

¹University of Liverpool, Liverpool, UK. ²University of Manchester, Manchester, UK
Division of Immunology, University of Liverpool, Duncan Building, Liverpool, L693GA

It has been shown previously that glycosylation can play an important role in IgE binding to allergens. Thus native and recombinant allergens, with differential glycosylation patterns have been shown to exhibit differential IgE binding capacity. Native and Recombinant forms of the iron binding protein lactoferrin (NLF and RLF) are available that have identical amino acid sequences, but are differentially glycosylated. In the current series of experiments we have compared the intrinsic ability of both forms of lactoferrin to induce IgG and IgE antibody responses following systemic exposure to BALB/c strain mice in the absence of adjuvant.

BALB/c strain mice received 250 μ l by intraperitoneal injection (ip) of various doses of RLF (1 to 5% w/v) or NLF (0.025 to 1% w/v) on day 0 and 7 and mice were exsanguinated on day 14. Sera were analysed for IgG antibody by enzyme linked immunosorbent assay (ELISA) using both forms of LF as substrate. IgE content of sera was analysed by homologous passive cutaneous anaphylaxis assay (PCA).

Immunisation with RLF alone (1 to 5%) induced detectable IgG responses, however only treatment with 5% RLF stimulated detectable, albeit low titre IgE antibody. In comparison, administration of NLF (1% to 0.05%) provoked relatively vigorous IgG and IgE responses. Equivalent IgG and IgE titres were achieved using 1% RLF and 0.025% NLF and 5% RLF and 0.025% NLF (a 40 fold and a 200 fold difference in protein concentrations respectively, for IgG and IgE antibody responses). Similar titres were achieved regardless of the form of lactoferrin used in the analytical phase.

In subsequent experiments, the impact of co-administration of both forms of lactoferrin on the induction of antibody responses was investigated. Combination of NLF (0.2%) and RLF (1%) resulted in a marked down regulation of anti-lactoferrin IgE antibody production, Thus a titre of 1 was achieved following immunisation with the combination of NLF and RLF whereas administration of NLF alone, resulted in high titre IgE antibody (1/16). The effects on IgE antibody production were allergen-specific. Thus RLF was without effect when co-administered with the unrelated allergen ovalbumin, with anti-ovalbumin IgE titres of 1/4 achieved regardless of the presence of RLF. Furthermore, using the same experimental design co-administration of RLF was without effect on the relatively vigorous IgE responses provoked by the peanut allergen peanut lectin, (titres of 1/16 recorded regardless of the presence of RLF).

The selective inhibition of anti-lactoferrin IgE responses suggests that RLF is influencing the induction phase of IgE antibody responses. These results indicate that the differential glycosylation patterns of the protein impact on the uptake, processing and/or presentation of antigen in such a way to skew towards a type 2 response and conditions that favour the production of IgE antibodies.

PROTEIN BODIES AND INTACT SOLUBLE PROTEIN FROM DIGESTED PEANUT ARE TRANSPORTED ACROSS THE PRE-SENSITISED GUT BY M CELLS

S. J. Chambers, M.S.J. Wickham, M. Regoli, E. Bertelli, P. A. Gunning, C. Nicoletti
Institute of Food Research, Norwich Research Park, Norwich, UK

Although alternative routes of sensitisation to peanut protein have been proposed, the gut immune system is likely to play a pivotal role in peanut allergy. However, the exact route of transport of immunogenic protein across the epithelium remains largely unknown. The aim of this study was to identify the route through which protein bodies and soluble proteins from digested peanuts penetrated the pre-sensitised gut epithelium.

In these experiments peanuts were subjected to an *in vitro* digestion procedure. Light microscopy analysis showed that peanut cells stored a large number of protein bodies and that subsequent to gastrointestinal digestion these were released following the disruption of the cell wall. The digested material was introduced into murine isolated gut loops for 30 minutes before tissues were recovered.

Transport of protein was monitored by transmission electron (TEM) and fluorescence microscopy. Initial investigations with low resolution fluorescence staining identified that protein bodies were associated with UEA-1+ M cells. TEM analysis identified protein bodies within M cells and also the presence of soluble peanut protein within intercellular spaces in the follicle-associated epithelium. Soluble peanut protein was also observed at the apical area, including microvilli, and cytoplasm of M cells but not in the adjacent enterocytes. Within the M cell the peanut protein appears to be associated with pockets containing lymphocytes.

In summary, gastrointestinal digestion of peanuts released a large number of protein bodies that are exclusively transported across the follicle-associated epithelium by specialised antigen-sampling M cells and delivered to the lymphoid tissue of Peyer's patch. Intracellular transport of soluble protein also occurred almost exclusively via M cells. We hypothesize that these conditions, which are known to favour strongly the induction of immune response rather than oral tolerance, are important in the genesis of allergic reaction to peanuts.

OLEOSINS: NEW AND IMPORTANT ALLERGENS IN LEGUMES, NUTS AND SEEDS?

L.Zuidmeer¹, M. Winter¹, J.Akkerdaas¹, S.Versteeg¹, C.Summers², A. Knulst³, V. Brettlova⁴, M. Wensing³, P. Schilte⁵, R. van Ree¹.

Laboratory of Allergy, Department of Experimental Immunology, Academic medical Center, Meibergdreef 9, 1105AZ Amsterdam

Background: Patients clinically reactive to peanut, tree nuts and/or seeds can score negative in various diagnostic tests. Oleosins are oilbody-associated proteins that have been tentatively implicated in allergic reactions to peanut, hazelnut and sesame seed.

Aim: To isolate natural oleosins from various sources and determine their allergenicity to nut and seed allergic patients as well as define their presence in commercially available extracts.

Methods: His-tagged hazelnut oleosin isoforms were expressed in *E. coli* and purified by affinity chromatography. IgE binding was tested by immunoblotting. Protein fractions enriched for oleosins were isolated from hazelnut, peanut, cashew, walnut and sesame with a combination of existing protocols. Main bands in the peanut, walnut and hazelnut fractions were identified with in-gel trypsin digestion followed by mass-spectrometry (MS). IgE binding to the enriched oleosin fractions was tested by RAST, CAP and/or immunoblot with serum from patients with (tree)nut-ingestion related history (n=185). Commercially available skin-prick test (SPT) materials for hazelnut and walnut were supplemented with the enriched oleosin fractions and selected sera were tested for IgE-reactivity to the (spiked) extracts on immunoblot.

Results: Semi-purified natural oleosins were poorly soluble and thus could so far not be further purified. Major bands of 27(H1), 24 (H2), 18(H3) and 14(H4) kDa were analysed by MS. H1 corresponded with an unknown protein with minor quantities of both oleosin isoforms, H2 with 11S globulin, H3 and H4 with two oleosin isoforms. 118/185 patients with (tree)nut ingestion related symptoms reacted to the enriched hazelnut oleosin fraction with RAST (>0.3 IU/ml). Both recombinant hazelnut oleosin isoforms inhibited IgE-binding to this fraction on blot. The enriched hazelnut oleosin fraction induced histamine release in basophils using allergic patient sera (n=16). Interestingly, the oleosin band seemed to be absent in (defatted) commercially available hazelnut/walnut extracts, and was clearly recognized in the spiked extracts.

Conclusion: Oleosins might play a role as an allergen with a high prevalence and properties to evoke systemic reactions. The association with oilbodies may protect oleosins from rapid proteolysis after ingestion. Due to extensive defatting of extracts used for *in vitro* diagnostics, oleosin-specific IgE can easily be missed.

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