PS 745 THE CONTRIBUTION OF PEANUT PROTEINS ARA H1, 2, 3 AND 6 IN PEANUT ALLERGY.

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Peanut is a major allergenic food product, and contains a large number of potentially allergenic proteins. The present study investigated the relative contribution of, and cross-reactivity between the major allergens Arah1-6 in a mouse model for peanut allergy. Mice were immunized by gavage with peanut protein extract or purified allergens Arah1-6. Hereafter, mice were challenged with the individual allergens and blood was collected to measure allergen-specific antibodies and the degree of mast cell degranulation (MMCP-1 and histamine). Spleens were isolated to measure allergen-specific T-cell reactivity. Sensitisation with whole peanut extract induced Arah1-6 specific IgE, IgG1 and IgG2a. In addition, sensitisation with the individual peanut allergens elicited antibody responses with specificity to the allergen used, but limited cross-reactivity between the allergens was observed. T-cell cultures showed Th1 and Th2 type cytokine production upon re-stimulation with both peanut extract and the individual peanut allergens, but cross-reactivity was observed only between Arah2 and 6. Remarkably, only Arah2 and 6 were able to elicit mast cell degranulation after oral challenge. In conclusion, individual peanut allergens do not significantly differ in their capacity to sensitize via the intestinal route, induce limited cross reactivity, but differ substantially in their capacity to cause effector responses. This implicates that peanut proteins may differ in clinical responses and may differ in efficacy for therapy in the human situation. In future studies, the mechanism behind the functional differences of individual peanut allergens and the protein cross-reactivity on T-cell and antibody level will be investigated *in vitro*.

PS 746 CHARACTERIZATION OF THE ALLERGENICITY OF DIFFERENT FORMS OF LACTOFERRIN.

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With the advent of genetically modified crop plants there is an increased interest in the development of methods for the identification of novel proteins as potential allergens. Current methods include assessment of resistance to digestion by simulated gastric fluid (SGF) and homology searches. There is a need for more holistic methods for the characterization of potential protein allergens and particularly the impact of production of the protein in novel expression systems that may result in differential posttranslational modifications such as glycosylation. The allergenicity (IgE inducing) and immunogenicity (IgG inducing) properties of wild type native (milk) human lactoferrin (NLF) and recombinant LF (rLF) produced in rice have been assessed. These forms of LF have identical amino acid sequences, but different glycosylation patterns: NLF has a complex glycoprofile including sialic acid and Lewis (Le) x structures, whereas the rLF form is far simpler and rich in mannose residues. Both proteins are very labile to digestion by SGF, with intact protein disappearing after 1min of incubation in the presence of pepsin at pH1.2. Antibody responses induced in BALB/c strain mice by intraperitoneal exposure to NLF and rLF were characterized. Sera were analysed for protein specific IgG and IgE by enzyme-linked immunosorbant assay (ELISA) and homologous passive cutaneous anaphylaxis assay (PCA), respectively. Immunisation with NLF stimulated vigorous IgG and IgE antibody responses, whereas rLF was 40-fold less immunogenic and 200-fold less allergenic, irrespective of endotoxin content. The glycans did not contribute to epitope formation, with equivalent IgE and IgG binding recorded for high titer anti-NLF antisera whether the immunising NLF or the rLF was used a substrate in the ELISA or PCA analysis. Similarly, identical low IgG and IgE anti-LF titers were recorded when either the immunising rLF or the NLF was used in the analysis of anti-rLF antisera. These data demonstrate that differential glycosylation profiles can have a profound impact on protein allergenicity.

PS 747 EXPOSURE TO TRICLOSAN AND BISPHENOL A AUGMENT ALLERGIC RESPONSES IN A MURINE MODEL OF ASTHMA.

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During the past several decades there has been a remarkable and unexplained increase in the prevalence of asthma. While the hygiene hypothesis provides one potential explanation, individuals in industrial societies are also inadvertently exposed to an increasing number of chemicals. While many chemicals are known to directly induce asthma there is also the potential for non-sensitizing chemicals to augment the immune response induced by other allergens. Our lab has previously demonstrated that dermal application of the environmentally relevant chemical, perflourooctanonic acid, simultaneously with exposure to a single concentration of protein allergen (OVA) was found to augment the allergic response to that allergen. Triclosan and Bisphenol-A (BPA) are widely used chemicals found in both occupational and public environments which have recently been associated with increases in allergy and asthma. BPA, considered to be non-sensitizing, is a substrate of polycarbonate plastics and has been produced in increasingly large quantities since the 1950s. BPA is used to form plastic bottles, as a lining for food and beverage cans, and as a flame retardant. Triclosan is an antibacterial compound that has been used in consumer products for 40 years and is currently found in many hand sanitizers and lotions. The tolerability and safety of triclosan has been evaluated in human volunteers with little indication of toxicity or sensitization. For these studies a murine model of asthma was used to evaluate the immunomodulatory effect of co-exposure to BPA or triclosan with OVA. Co-exposure to each of these chemicals individually (as low as 30% BPA and 1.5% triclosan) with OVA resulted in an at least a 2-fold increase in OVA-specific IgE and an augmentation of the airway hyper-reactivity response to methacholine challenge (as low as 7% BPA and 0.75% triclosan) as compared to OVA exposure alone. Understanding the mechanisms by which mixed exposures influence and augment asthma and asthma-like symptoms may lead to better prevention strategies for those at risk for asthma.

PS 748 IMPACT OF DOSING FREQUENCY ON ANAPHYLAXIS IN CYNOMOLGUS MONKEYS ADMINISTERED TRU-015, AN ANTI-CD20 SMIP™ THERAPEUTIC.

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TRU-015, a chimeric (mouse and human) anti-CD20 SMIP™ (mono-specific protein therapeutic), was administered intravenously (IV) to male and female cynomolgus monkeys at 0 (vehicle), 15, 50, and 150 mg/kg/cycle (4 to 7 animals/sex/group) for 6 cycles (1 dose/2 wks) to evaluate its toxicity. Infusion reactions consistent with anaphylaxis occurred within 1 hr of dosing starting with 1 male at 15 mg/kg after the 3rd dose. By the 5th dose, animals at all dose levels were affected with an inverse dose relationship in the severity of reactions. Diphenhydramine pretreatment initiated at the 6th dose had no apparent impact on anaphylaxis, leading to dosing termination. A total of 9 of 36 TRU-015-dosed animals were affected, with elective euthanasia of 2 animals at 15 mg/kg. Of the 9 animals affected, 5 had anti-drug antibodies (ADA) and 4 had evidence of complement activation. The infusion reactions, onset of reactions relative to dosing, presence of ADA, and signs of complement activation were consistent with anaphylaxis secondary to an immune response to TRU-015. In an effort to successfully conduct a chronic toxicity study, and based on shorter term toxicity studies in which weekly dosing did not cause infusion reactions, TRU-015 was administered IV at 0 (vehicle), 15, and 150 mg/kg/cycle (4 animals/sex/group) for 13 cycles (1 dose/wk). With this regimen, only 1 animal at 15 mg/kg out of 16 TRU-015-dosed animals had an infusion reaction consistent with anaphylaxis. The reaction occurred within 1 hr of the 7th dose and led to this animal's death. This animal also had ADA and evidence of complement activation. Therefore, increasing the dosing frequency successfully reduced the incidence of anaphylaxis related to TRU-015 administration. This new dosing regimen permitted the conduct of longer duration toxicity studies in NHPs. Similar immune reactions have not been observed in humans following extensive clinical experience.

PS 749 A CUTANEOUS HYPERSENSITIVITY REACTION TO A THYROID HORMONE SUPPLEMENT IN A DOG.

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A dog with clinical signs of hypothyroidism (an autoimmune disease common to dogs and humans) and low thyroid hormones (T4 and its T3 metabolite) received a generic T4 supplement. After 2 weeks, the dog developed a severe pruritus and pyoderma on its neck and back with a wide spread erythema. The dog responded to supplement withdrawal, antibiotics and steroids. A different brand of T4 supplement was prescribed, but the dog had a more severe reaction within less than 48h. The new drug was therefore discontinued, and the skin signs resolved within a few days. This led to a clinical diagnosis of delayed immune-mediated skin reaction to the active ingredient of the supplement.

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