63 Comparison of Th1 and Th2 Immune Responses in *Chlamydia pneumoniae*-infected Peripheral Blood Mononuclear Cells (PBMC) of Adult and Pediatric Asthmatics

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RATIONALE: *C. pneumoniae* is a common cause of upper and lower respiratory tract infections in both children and adults and can cause asthma exacerbations. Our laboratory has previously demonstrated that T-lymphocyte memory responses of PBMC infected with *C. pneumoniae* in vitro was increased in asthmatics compared to healthy subjects without asthma. However, there were both Th1 and Th2 responses present in the asthmatic population. In this investigation, we studied differences in the Th response (Th1 versus Th2) to *C. pneumoniae* infection of PBMC between pediatric and adult patients with asthma.

METHODS: PBMC ($1x10^6$/ml) from pediatric (<18y) (n=15) and adult (>=18y) (n=9) asthmatics were infected or mock infected with *C. pneumoniae* CM-1 and cultured for 48h. Levels of IL-4 and IFN-gamma in supernatants were measured by ELISA. Cytokine levels of mock infected samples were subtracted from those of infected samples for each study subject. Differences between age groups were determined by Mann-Whitney Test.

RESULTS: PBMC from asthmatic adults produced more IL-4 in response to *C. pneumoniae* stimulation than those from asthmatic children ($p<0.01$). There was no significant difference in IFN-gamma production after *C. pneumoniae* infection between the two study groups ($p=0.9$).

CONCLUSIONS: We found that a *C. pneumoniae*-induced Th2 immune profile was prevalent in adult asthmatics, but not in pediatric asthmatics. Repeated or persistent infections with *C. pneumoniae* can occur and repeat exposure may lead to change in Th response with increasing age in predisposed individuals. Whether this age-specific response to *C. pneumoniae* contributes to the symptomatology of asthma remains to be further elucidated.

64 Diversity and complexity of mouse allergens in allergenic products assessed with an immuno-allergomic approach

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RATIONALE: Mouse allergy is common among laboratory animal workers and infested homes. The predominant allergen, Mus m 1, is part of the Mouse Urinary Proteins (MUPs) complex. The full diversity and immunogenic potential of MUPs have not been fully established yet, and it is not clear that MUPs are the only relevant sensitizing agents. This work investigates the diversity and relative immunogenicity of various MUPs.

METHODS: Liquid chromatography/mass spectrometry (LC/MS) was used for deep proteomic analysis of mouse urine and epithelial extracts. Mouse proteins, resolved by two-dimensional polyacrylamide gel electrophoresis, were screened against IgG and IgE. Reactive spots were picked and examined using LC/MS. Finally, a multiple reaction monitoring (MRM) method was developed for total MUP as well as isoform-specific quantification. LC/MS and MRM results were compared to Mus m 1 quantification by ELISA.

RESULTS: We established a global proteomic signature and a detailed profile of MUPs including their post-translationally modified variants in commercial products and in collected mouse urine. MUP3 resolved into four different protein forms while MUP19 appears to have three protein variants. Using bioinformatics and sequence analyses, we identified unique tryptic peptides for 10 MUPs. Isotopically-labeled unique reference peptides were chemically synthesized and used to develop an MRM method for isoform-specific quantification of MUPs. The MRM assay is being evaluated for comparability with various ELISA assays of Mus m 1.

CONCLUSIONS: We established molecular and immunogenic fingerprints of mouse epithelial and urinary allergenic products. The results are key in guiding research, characterization, quality control, and standardization of existing and future products.

65 Cat Natural Exposure Chamber (NEC™) Rhinoconjunctvitis Study

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RATIONALE: Cat dander is a common cause of allergic rhinitis. A cat exposure room with allergen concentrations similar to homes with cats can provide a relevant model to test allergy medications. We assessed the reproducibility of the clinical response in cat allergic and non-allergic subjects, in a natural exposure chamber (NEC).

METHODS: Six subjects (4 allergic and 2 non-allergic) underwent 2 challenges, one week apart. During the challenges cat bedding was shaken every 15 minutes; nasal, ocular and respiratory symptoms were captured every 5 minutes using a 4-point severity scale and spirometry was performed every 15 minutes outside the NEC. Aerosolized Fel d1 sampled by portable pumps (Gilian 5000) was measured at 3 room locations and from a sampler worn by the subject. Fel d1 was also measured from swabs obtained from walls and the floor.

RESULTS: Room Fel d 1 was 189.5 ± 183.4 ng/m^3; personal samplers reported 63.4 ± 51.2 ng/m^3. Rhinoconjunctivitis symptoms for the two challenges in the 4 cat-allergic subjects were similar (6.5 ± 1.7 vs. 6.25 ± 3.36). Nasal symptoms predominated (5.25 ± 2.1, Challenge 1; 4.0 ± 2.4, Challenge 2). Allergic subjects reported modest respiratory discomfort during the challenges although none experienced a fall in FEV_1. The 2 non-cat allergic subjects experienced no nasal, ocular or respiratory symptoms.

CONCLUSIONS: The NEC maintains airborne Fel d1 levels similar to homes and induces symptoms in allergic subjects while non-allergic subjects remain asymptomatic. Rhinoconjunctivitis and respiratory symptoms were comparable in two sessions separated by a week indicating that the NEC would be suitable for evaluating the effects of treatment.

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