

224 Age Difference in the Immune Response to Endotoxin (LPS) Shapes Th2-Mediated Airway Inflammation and Development of Asthma.



Beatriz León, PhD, and Andre Ballesteros-Tato; University of Alabama at Birmingham, Birmingham, AL.

RATIONALE: In addition to genetic factors, environmental exposures is also of primary importance for the development of asthma and allergy disorders in children, as demonstrated by epidemiological data showing children growing up in traditional farms seem to develop asthma less often than children growing up in urban areas. According to the hygiene hypothesis, this is due to increased exposure to endotoxin (LPS) and other farm-related microorganism-derived compounds. However little is known about how early-life contact to microbial compounds influence the development of asthma.

METHODS: Adult (8 weeks-old) and infant (3 weeks-old) mice were intranasally (i.n.) sensitized with 25µg of house dust mite (HDM) allergen extract in the presence of different doses of LPS, for 3 consecutive days. 3 weeks later, mice were i.n. challenged with 25µg of HDM extract for 3 consecutive days.

RESULTS: HDM allergen sensitization in adult mice induced T-helper type 2 (Th2)- driven airway inflammation, i.e., influx of Th2 cells into the airways, airway eosinophilic inflammation and increased IgE-producing plasmablasts. In contrast, HDM allergen sensitization with low-dose LPS (10 µg) abrogated HDM-driven Th2-mediated allergic airway inflammation without increasing Th1 cell trafficking into the airways. Unlike adults, infant mice exposed to HDM with low-dose LPS (10 µg) developed Th2 responses allergic airway inflammation and required allergen sensitization with high-dose LPS (100 µg) to effectively suppress allergen-specific Th2 responses and allergic inflammation.

CONCLUSIONS: These results show that airway exposure levels of endotoxin provide different protection against asthma and allergies in adults and infants.

225 Peanut Specific-CD4+ T Cells Responses in Peanut Allergic and Peanut Sensitized but Tolerant Subjects



William W. Kwok, PhD¹, Mary L. Farrington, MD², Elizabeth Whalen, PhD³, Erik R. Wambre, PhD, MBE³, Kari Nadeau, MD, PhD⁴, and Amedee Renand, PhD⁵; ¹Benaroya Research Institute at Virginia Mason, Seattle, WA, ²Virginia Mason Medical Center, Seattle, WA, ³Benaroya Research Institute, Seattle, WA, ⁴Pediatric Allergy Immunology, Stanford University School Medicine, Stanford, CA, ⁵Benaroya Research Institute at Virginia Mason, Seattle.

RATIONALE: The immunogenicity of different Ara h components in eliciting specific CD4 T cell responses in both peanut allergic and peanut sensitized but tolerant subjects (i.e. presence of peanut specific IgE but non-reactive to peanut food challenge) remains unclear.

METHODS: Fourteen allergic and six tolerance subjects were recruited. T cell responses toward Ara h 1, 2, 3, 6 and 8 were evaluated by CD154 up-regulation assays. T cell responses towards Ara h 2 were also evaluated by tetramer staining assay.

RESULTS: T cell responses against Ara h 1, 2, 3 and 6 were significantly stronger compared to response against Ara h 8 in allergic subjects. Phenotypes of Ara h 2- and Ara h 1, 3, 6-specific-T cells were heterogeneous and could be CCR4+CD27-CRTH2+, CCR4+CD27+CCR6+ or CCR4+CD27+CCR6-. Experiments with both CD154 up-regulation and tetramers staining assay in evaluating Ara h 2 responses gave similar outcomes. Peanut-sensitized but tolerance subjects had significantly lower frequencies of Ara h 2 and Ara h, 1, 3, 6-specific T cells compared to allergic subjects. Ara h 8-specific responses in most subjects of this group were very weak or absence.

CONCLUSIONS: Specific memory CD4 T cell response with a predominant TH2 phenotype against Ara h 1, 2, 3 and 6, but not against Ara h 8, was related to an allergic response. Conversely, a weak or an absence of a

specific memory CD4 T cell response against Ara h 1, 2, 3 and 6 was related to an absence of a clinical response in peanut sensitized but tolerant subjects.

226 Hippocampus reidi, a Marine Natural Product Reduces TH2 Cytokine Levels and Attenuates NF-KB Expression



Hugo Bernardino Ferreira Silva, Sr¹ Raimon RIOS Silva¹, Norma Vilany Queiroz Carneiro², Anaque Oliveira Pires³, Marta Santos Serafim Machado⁴, Cíntia Rodrigues Marques², Ryan Santos Costa², Tamires Carneiro Brasil³, Paulo Pessoa ALBUQUERQUE Filho⁵, Eryvelton Souza Franco⁵, Maria Bernadete Souza Maia⁵, Rosana Beatriz Silveira⁵, Neuza Maria Alcântara-Neves², and Camila Alexandrina Figueiredo²; ¹Federal university of Bahia, Salvador, Brazil, ²Institute of Health Sciences, Federal University of Bahia, Salvador, Brazil, ³Federal University of Bahia, Salvador, Brazil, ⁴Bahia Adventist College, Cachoeira, Brazil, ⁵Federal University of Pernambuco, Recife, Brazil.

RATIONALE: *Hippocampus reidi* (seahorse) is widely used in Brazilian folk medicine to treat asthma and other inflammatory conditions. The aim of this study was to describe the immune modulatory potential of the *H. reidi* hydroalcoholic extract in vitro.

METHODS: Spleen cells (5×10^5 in each well) of BALB/c mice were stimulated with 2,5 µg/ml of mitogen (PWM), and exposed or not to *Hippocampus reidi* hydroalcoholic extract (EHR) at different concentrations (125, 62, 31 µg/ml) during 72 hours at 37°C and 5% CO₂. The cytokine levels from cell supernatants were measured by ELISA. Activation NF-kB was assessed by RT-PCR.

RESULTS: Our results have shown that PWM increased the levels of IL-4 and IL -5, compared with non-stimulated cultures. Treatment with EHR decreased PWM-induced IL-4 production at all concentrations tested (125 and 62 µg/mL, (p 758.9 ± 86.6 vs 364.7 ± 43.7; 561 ± 47.65, ***p<0.001) and 31 µg/mL, (758.9 ± 86.6 vs 641.1±83.9 *p <0.1); as well as IL-5 production at 125 µg/mL (441.4 ± 154.8 vs 212.3 ± 67.4, ***p <0.001) and 62 µg/mL (441.4 ± 154.8 vs 235.6±74.7 *p<0.1). PWM-stimulated spleen cells had increased expression of NF-kB compared to control cultures (p < 0.001). However, when the cells were stimulated with PWM and treated with *H. reidi* (125µg/mL) the expression of NF-kB was reduced (1.68 ± 0.20 vs 0.25 ± 0.08, ***p <0.001), in comparison to the positive control.

CONCLUSIONS: The findings presented herein demonstrate that HER was able to modulated Th2 cytokines levels and attenuates NF-kB gene expression in vitro.