IgE levels could be recorded, whereas immunizations with OVA-AAVLP rendered background IgE levels only. In accordance, sera of OVA mice which permitted mast cell degranulation upon OVA trigger in a specific β-hexosaminidase release assay, whereas sera of OVA-AAVLP mice did not contain anaphylactogenic antibodies. In an in vivo anaphylaxis experiment, upon intravenous OVA challenge OVA-immunized mice presented significant drop of body temperature, whereas AAVLP-OVA mice remained unaffected. 

**Conclusions:** Our study demonstrates the immunogenicity, safety and efficacy of AAVLP as display system of B-cell epitopes for vaccination.

---

**105**

**M-Cell Targeting by Neuraminidase Functionalized Microparticles for Future Application in Oral Immunotherapy**

Susanne C. Diesner,1 Cornelia Schultz,2 Xueyan Wang,3 Gerda Ratzinger,1 Philipp Stark,3 Vera Assmann,2 Kristina Kreiner,1 Franziska Roth-Walter, PhD,2 Isabella Pali-Schöll, PhD,3 Eva Azöls,2 Franz Gabor,1 and Eva Untersmyer1,3 . Department of Pathophysiology and Allergy Research and Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Vienna, Austria; 1Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna; 2University of Vienna, Department of Pharmaceutical Technology and Biopharmaceutics, Vienna, Austria.

**Background:** Recently, we demonstrated in an experimental mouse study that mucosal M-cell targeting with Aloe vera auctoria lectin (AAL) coated Poly (D,L-lactide-co-glycolide) (PLGA) microparticles represents a promising oral treatment approach in IgE mediated allergy. Due to its structural similarities with AAL we aimed to assess Neuraminidase (NA) from *Fibrochlorella* as a novel M-cell specific targeters and compared its properties to AAL and wheat germ agglutinin (WGA) representing 2 plant lectins, which target either α-L fucose or N-acetyl-d-glucosamine (GM1).

**Methods:** The resistance against gastric digestion of NA, AAL and WGA was analyzed in simulated gastric fluid (SGF) experiments. Intestinal epithelial binding was determined using the colon carcinoma cell line Caco2, which represents a well established model for the human intestinal epithelium. Binding specificity was evaluated by inhibition experiments by incubating Caco2 cells with Biotin-labeled NA, AAL or WGA, after preincubation with α-L fucose, monogansyllose (GM1) or N,N,N′-triacetyl-chitotriose (TCT). The stimulatory effects of the binding substances on the intestinal microenvironment were investigated by cytokine read-out experiments in real-time PCR. Further, the transethelial uptake of NA, AAL- or WGA-functionalized fluorophos was evaluated in a human M-cell co-culture model.

**Results:** All 3 targeters were stable up to 180 minutes in SGF, indicating their suitability for oral application. The binding partners were α-L fucose for AAL and TCT for WGA, whereas NA interacts with intestinal epithelial cells via α-L fucose and additionally GM1. NA skewed the cytokine production by inducing a 2-fold increase of the Th1 cytokine IFNγ after 60 minutes, whereas AAL decreased the overall cytokine expression. In a human M-cell co-culture model, a higher transepithelial transport rate of fluorophos coated with NA and AAL was observed as compared to WGA and plain particles. 

**Conclusions:** NA specifically targets M-cells via α-L fucose and additionally GM1 and, thus, increases the transepithelial transport of NA coated particles. Due to the immunomodulatory capacity on intestinal epithelial cells, NA functionalized microparticles may represent a promising M-cell specific targeting approach for oral immunotherapy.

---

**106**

**Neisseria Meningitidis Derived Proteoliposome as an Adjuvant for Allergen Vaccines**

Wendy Ramirez González, MSc,1 Alexis Labrada, PhD,2 Virgilio Bourg, MSc,2 Bárbara González, PhD,4 Damarys Torralba,4 Arélis Más Quintero, MSc,5 Oliver Pérez, PhD,3 and Miriam Lastre, PhD.4 Allergens Laboratory; 2Allergens, and; 3Department of Biological Tests, National Center of Bioproducts, Havana, Cuba; 4CENPALAB, Havana, Cuba; 5Finlay Institute, Havana City, Cuba. 

**Background:** In recent years one important trend of Allergen-specific immunotherapy is to investigate new adjuvants with immunomodulatory properties. The outer membrane vesicle or proteoliposome (PL) from *Neisseria meningitidis* serogroup B has been reported as a potent adjuvant inducing a Th1-skewed response. The aim of this work was to assess the immunogenicity of a novel anti-allergic vaccine candidate based on purified allergens from *Dermatophagoides pteronyssinus* mite and PL as adjuvant, both components adsorbed onto Aluminum hydroxide.

**Methods:** In a preventative experimental setting BALB/c mice were administered with 3 doses containing 5 μg of Der s 1 allergen at one week intervals by subcutaneous route. Further, mice were subjected to allergen challenge by aerosol inhalation. In another experiment, mice were administered first with 2 doses of PL + Alum and later with the whole vaccines formulation, including the allergen. The allergen-specific antibody response was assessed determining serum levels of IgE, IgG1, and IgG2a by ELISA. The local allergic inflammatory response was evaluated by measuring cytokine levels (IL-4, IL-5, IFNγ and IL-10) in broncho-alveolar lavage (BAL) by ELISA.

**Results:** The formulation consistently induced IgG2a, as well as IgG1 antibodies with a potential anti-IgE blocking effect. The induction of IgG2a was clearly PL dependent while IgG1 was dependent mostly of Alum. Prior administration of the proteoliposome with alum without allergen showed to enhance this allergen-specific immunogenic effect. The vaccine prevented the development of systemic (IgE) and local allergic response in mice subjected to allergen exposure by inhalant route. Vaccinated mice showed lower levels of serum IgE. Th2 cytokines (IL-4, IL-5) in BAL and lower eosinophil counting in blood as compared to controls. Histological examination of lungs showed also a diminished allergic inflammatory response in vaccinated mice in contrast with mice which were administered with the conventional formulation of Alum-adsorbed allergen.

**Conclusions:** The antiallergic protective effect was proven in a preventative setting, showing to decrease the inflammatory response in the lungs of mice exposed to allergen aerosol, as well as, a Th2-antagonistic immune response with few injections.

---

**107**

**Immunomodulatory Effects of Manumycin-type Antibiotics on Human Macrophages**

Ilja Striz, MD, PhD,1 Eva Brabcova, MSc,1 Katerina Petrickova, PhD,2 Libor Kolesar, MSc,1 Eliska Thorburn, MSc,1 Marcela Jaresova, MSc,1 Alena Sekerkova, MSc,1 and Miroslav Petricek, PhD.2,3 Institute for Clinical and Experimental Medicine, Prague, Czech Republic; 2Institute of Microbiology, Academy of Sciences of Czech Republic, Prague, Czech Republic. 

**Background:** Polyketide-derived antibiotics including macrolides are known to exert potent anti-inflammatory and immunomodulatory effects beyond their purely antibacterial action. The mechanisms of their biological activities are still being investigated but the effect on signalling pathways of transcription factors which regulate a number of pro-inflammatory and/or pro-fibrotic genes might be preferentially involved. The aim of our study was to assess the effect of manumycin and structurally related compounds asukamycin and collabomycin on a release of proinflammatory cytokines IL-1β and IL-18 from THP-1 monocyte/macrophage cell line. Furthermore, the level of mRNA expression of multiple genes associated with immune regulation has been studied.

**Methods:** The THP-1 cells were cultured in RPMI1640 with 5% fetal calf serum and then stimulated with TNF alpha (20 ng/mL) under serum free conditions in the presence or absence of manumycin and asukamycin (both at 0.3 μg/mL). The concentrations of cytokines in culture supernatants were measured by ELISA (IL-18, MBL) or Luminox (IL-1 beta, R&D). Quantitative RT-PCR (SABiosciences) was used for the evaluation of 84 different gene expressions in TNF alpha and manumycin stimulated cultures.
Results: IL-1 beta was not detectable in culture supernatants of unstimulated THP-1 cells but appeared in response to TNF alpha (4.96 + 0.59 pg/mL). Both manumycin (0.34 + 0.48 pg/mL) and asukamycin (1.06 + 0.81) inhibited IL-1 beta release induced by TNF alpha. IL-18 was found to be constitutively produced (14.68 + 7.83 pg/mL) and the release was doubled by TNF alpha (30.98 + 2.21 pg/mL) and inhibited to basal values by both manumycin (18.04 + 10.21 pg/mL) and asukamycin (12.96 + 2.32 pg/mL). Manumycin inhibited mRNA expression of several genes associated with proinflammatory responses including IL-1 beta, IL-6, and TLR8. Among the genes upregulated in response to manumycin, HMox1, gene for heme oxygenase 1, showed the highest mRNA induction.

Conclusions: We assume from our study that manumycin and asukamycin represent potent inhibitors of IL-1 beta and IL-18 release from human macrophages. Some of the potentially proinflammatory genes are regulated on the level of transcription.

Supported by MSMT grant 2B06154.

INDOOR RISK FACTORS FOR ASTHMA

108 The Relationship of Pets, Vitamin D and IGE Concentrations to Upper Respiratory Infections in the First Year of Life
Dennis Ownby, MD,1 Edward Peterson, PhD,2 Ganesa Wegienka, PhD,2 Susan Lynch, PhD,3 Homer Boushey, MD,4 Nicholas Lukacs, PhD,3 Edward Zoratti, MD,6 Suzanne Havstad, MA,2 Kevin Bobbitt, PhD,2 Kimberley Woodcroft, PhD,2 and Christine Cole Johnson1,4,5

Pediatrics, Section of Allergy, Immunology, Rheumatology, Georgia Health Sciences University, Augusta, GA; 2Public Health Sciences, Henry Ford Hospital & Health System, Detroit, MI; 3University of California San Francisco, San Francisco, CA; 4Medicine, University of California, San Francisco, San Francisco, CA; 5Pathology, University of Michigan, Ann Arbor, MI; 6Allergy and Immunology, Henry Ford Hospital & Health System, Detroit, MI.

Background: The childhood origins of asthma are highly complex but viral respiratory infections during the first year of life may be associated with wheezing and later asthma risk. Recent studies have shown that both exposure to household pets and higher serum vitamin D concentrations may reduce wheezing illness in children.

Methods: To investigate potential relationships between household pet exposure, cord blood (CB) vitamin D and IgE concentrations and the number of upper respiratory infections (URIs) in the first year of life, we analyzed information from a geographically-based, prospective, non-high-risk, birth cohort. Household pets were assessed during pregnancy and medical records were abstracted for doctor visits of URIs. Because of large differences in vitamin D concentrations between Blacks and Whites racial stratification was done for some analyses.

Results: The cohort consisted of 1055 children of whom 62.4% were Black and 49.4% were female. When all children were considered, a one natural log unit increase in CB vitamin D concentration was associated with a greater risk of a URI visit (RR = 1.27, 95% CI, 1.01-1.59, P = 0.037) which remained after adjusting for the season of birth (RR = 1.28, P = 0.033). Individually adjusting for the number of children in the family, CB IgE, child gender, family or maternal smoking and race did not substantially change the association of vitamin D to URIs (all RR’s were 1.25-1.27), although the risks only remained statistically significant with CB IgE (P = 0.035) and gender (P = 0.043). When models stratified by race including pets, dogs only, or cats only, and CB IgE were fitted with the other variables, the relationship between CB vitamin D disappeared for whites but did not change in magnitude for blacks (RR = 1.31; 95% CI, 0.89-1.92; P = 0.165). Among Whites the only variable associated with URIs was a relationship with female gender (RR = 0.62, 95% CI, 0.41-0.94; P = 0.025) with being in daycare approaching significance (RR = 1.72, 95% CI, 0.94-3.14; P = 0.08).

Conclusions: In a large, prospective, non-high-risk birth cohort higher, CB vitamin D concentration, after adjusting for other potential confounding variables, was not associated with a decreased risk of physician diagnosed URIs in the first year of life.

109 Prevalence of Cockroach and Mouse Sensitization Among Children Hospitalized for Wheezing and Asthma
Terri Moncrief, MD,1 Andrew Beck, MD,2 Emily Greenberg, BA,2 Heather Strong, BA,3 Jeffrey Simmons, MD, MS,1 and Robert Kahn, MD, MPH2, 3Allergy/Immunology, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH; 2General and Community Pediatrics, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH.

Background: The prevalence and demographic correlates of cockroach (CR) and mouse sensitization among children hospitalized for wheezing and asthma are not known. Objectives: (1) To describe the prevalence of CR and mouse sensitization in a population-based sample; (2) To examine factors potentially associated with allergic sensitization including sociodemographic factors and asthma history.

Methods: We examined baseline data of the first 416 children enrolled in a prospective study cohort between August 2010 and February 2011. Eligible children were aged 1 to 16 years, were admitted for bronchodilator-responsive wheezing or acute asthma to a single children’s hospital that captures >90% of all asthma admissions in the county. Allergic sensitization was determined using specific IgE to CR and mouse. Caregivers were surveyed regarding sociodemographic characteristics and asthma history. Associations were assessed using chi-square statistics.

Results: The sample is 65% African-American, 76% publically insured. 78% report household income less than $60,000. 81% have a previous physician-diagnosis of asthma. 26% of children are sensitized to CR, 16% to mouse, and 34% are sensitized to either CR or mouse. 8% are sensitized to both. Patients younger than 4 years are less likely to be sensitized to CR (10 vs 34%, P < 0.0001) and mouse (8 vs 20%, P = 0.002) than older patients. Patients with a previous physician-diagnosis of asthma are more likely to be sensitized to CR (29 vs 13%, P = 0.007) and mouse (13 vs 9%, P = 0.06) than patients without a previous diagnosis. Compared to children in families with annual income >$90,000; those in families earning less than $15,000 were more likely to be CR sensitized (33 vs 18%, P = 0.01). The opposite trend exists for mouse sensitization: 13% of low income children are sensitized compared to 25% of high income children (P = 0.02).

Conclusions: In a population based sample, one-third of children admitted for bronchodilator-responsive wheezing or asthma are sensitized to either CR or mouse. Sensitization is associated with older age, a previous physician-diagnosis of asthma, and household income. Assessment of allergic sensitization during an inpatient admission may be an opportunity to target interventions for children at highest risk of allergic-related asthma morbidity.

110 Is Pet Ownership Associated with Higher Vitamin D?
Christine Cole Johnson,1 Edward Peterson, PhD,1 Ganesa Wegienka, PhD,1 Suzanne Havstad, MA,2 Kimberley Woodcroft, PhD,1 Kevin Bobbitt, PhD,1 Susan Lynch, PhD,3 Homer Boushey, MD,4 Nicholas Lukacs, PhD,3 Edward Zoratti, MD,6 and Dennis Ownby, MD,4 Public Health Sciences, Henry Ford Hospital & Health System, Detroit, MI; 2Medicine, University of California, San Francisco, San Francisco, CA; 3Pathology, University of Michigan, Ann Arbor, MI; 4Allergy and Immunology, Henry Ford Hospital & Health System, Detroit, MI; 5Pediatrics, Section of Allergy, Immunology, Rheumatology, Georgia Health Sciences University, Augusta, GA.

Background: Pet keeping has been linked with decreased risk of allergic sensitization, which has been associated with the Hygiene Hypothesis; and more recently, by ourselves and others, to particular home microbiome