232 Exaggerated Airway Wall Eosinophilia and Physiologic Dysfunction in Asthma-like Airways after Chronic *Alternaria* Exposure in Rats

R. L. Sorkness[1], K. M. Herricks[1], R. J. Szakaly[2], R. F. Lemanske, Jr.[2], L. A. Rosenthal[2]; [1]School of Pharmacy, University of Wisconsin, Madison, WI, [2]Medicine, University of Wisconsin, Madison, WI.

RATIONALE: Allergy to *Alternaria* (ALT) is a risk factor for asthma inception and asthma fatalities. We hypothesized that airways with pre-existing asthma-like pathology would be more vulnerable than normal airways to ALT allergen exposure.

METHODS: Rats with a chronic asthma-like condition (airway obstruction, hyperresponsiveness, and remodeling) were created by inducing a viral bronchiolitis at an early age, and were compared with sham-inoculated controls. After 8 wks recovery from viral illness, all rats were sensitized to crude ALT extract via s.c. injection. After baseline physiology studies (forced expiratory maneuvers, lung volumes, and lung elastic recoil in lightly anesthetized, intubated animals), rats were exposed to aerosolized ALT or PBS once weekly X6 wks. Physiology studies were repeated, and right lung bronchoalveolar lavage (BAL) and left lung quantitative histology were employed to evaluate inflammation 5 days after the final challenge.

RESULTS: Although both asthma-like and control rats had ALT-induced decreases in $FEV_{0.2}$/FVC ratio ($p<.05$), only the asthma-like rats had ALT-induced losses of lung elastic recoil ($p<.02$). The ALT-challenged asthma-like rats had marked increases in airway wall eosinophils ($p<.0001$), and increased goblet cells in small airways ($p<.01$), while no eosinophils were detected in BAL fluid. In contrast, ALT-challenged controls had only small numbers of eosinophils in both BAL and airway walls.

CONCLUSIONS: Pre-existing asthma-like airway pathology alters the response to ALT allergen, resulting in persistent eosinophilic airway wall infiltrates, goblet cell metaplasia, and reduced lung elastic recoil–all features of severe asthma in humans.

Funding: NIH NHLBI

233 Systemic Effects of Chronic Asthma on Bone Marrow Eosinophil Development

M. B. Hogan[1], D. Piktel[1], A. F. Hubbs[2], L. McPherson[2], K. S. Landreth[1]; [1]West Virginia University, Morgantown, WV, [2]NIOSH, Morgantown, WV.

RATIONALE: Chronic asthma is associated with pulmonary eosinophilia, increased mucous production and basement membrane thickening. It is unknown if systemic findings of asthma such as bone marrow eosinophilia continue in chronic disease. We utilized a murine model of chronic asthma to determine whether enhanced eosinophil production observed following allergen sensitization continues after prolonged allergen exposure.

METHODS: Four to six week old BALB/c or A/J mice received intranasal ovalbumin or saline 3 times/week for 12 weeks. Pulmonary eosinophilic peribronchiolar cuffing, mucous production and fibrosis were evaluated by a pathologist. Pulmonary fibrosis and mucous production were quantified by morphometry in A/J mice. Bone marrow CFU-eo and eosinophils were enumerated 24 and 72 hours after the last intranasal exposure.

RESULTS: Ovalbumin sensitized mice developed increased eosinophilic peribronchiolar cuffing ($p<0.001$) and mucous production ($p<0.001$) compared to controls. Only A/J mice developed significant peribronchiolar fibrosis ($p<0.01$). By morphometry, chronic ovalbumin exposure significantly increased mucous production ($p<0.02$) and peribronchiolar collagen deposition consistent with increased basement membrane thickening ($p<0.02$) in A/J mice. Significant bone marrow eosinophilia developed in A/J mice at 24 hours ($p<0.01$) and 72 hours ($p<0.001$). No significant differences were noted in bone marrow CFU-eo in ovalbumin exposed mice.

CONCLUSIONS: Chronic ovalbumin exposure increased mucous production and peribronchiolar eosinophils consistent with asthma in both mouse strains. Only A/J mice developed peribronchiolar fibrosis associated with chronic asthma. Systemic effects of chronic asthma with accelerated bone marrow eosinophil production were only noted in A/J mice which developed peribronchiolar fibrosis.

Funding: Department of Defense, American Lung Association

234 Molecular Markers of Eosinophilopoiesis at Birth: Kinetics of Cord Blood GATA-1, MBP and IL-5 Receptor Expression

A. K. Ellis, L. Crawford, J. A. Denburg; Division of Allergy & Clinical Immunology, McMaster University, Hamilton, ON, CANADA.

RATIONALE: Using colony assays and flow cytometry, we have recently shown that eosinophil/basophil (Eo/B) progenitor phenotype and function are associated with atopic risk at birth and infant clinical outcomes. The current study aimed to utilize real-time polymerase chain reaction (Q-PCR) to ascertain the kinetic patterns of expression of CB Eo/B-lineage specific genes, GATA-1, MBP and IL-5Rα in order to develop molecular markers of Eo/B differentiation.

METHODS: CB non-adherent mononuclear cells (NAMNCs) were isolated from random fresh and frozen samples, and incubated in the presence of rhIL-5 (1 ng/mL). At 24, 48 and 72h post-stimulation, RNA was isolated, reverse transcribed, and expression of IL-5Rα, GATA-1, and MBP were determined utilizing comparative Q-PCR in a multiplex reaction. The relative expression ratios between stimulated and un-stimulated cells were calculated using the delta-delta Ct method.

RESULTS: Stimulation with IL-5 resulted in an up-regulation of GATA-1 expression; this peaked between 24 and 48hrs. In contrast, MBP was up-regulated in a slowly progressive pattern, with maximal up-regulation at 72h, while there was a stable, minor down-regulation of IL-5Rα. In keeping with these molecular kinetic findings, Eo/B colony-forming cells, grown in 14-day methylcellulose culture, were found to be present in relation to timing of GATA-1 expression.

CONCLUSIONS: Multiplex Q-PCR analysis of mRNA from CB mononuclear cells stimulated with IL-5 demonstrates sequential expression of critical lineage-specific events, and can be used as a surrogate, molecular marker of CB Eo/B differentiation in clinical studies.

Funding: Canadian Institute of Health Research (CIHR) and Allergy, Genes & Environment Network of Centres of Excellence (AllerGen NCE)

235 Urokinase-type Plasminogen Activator (uPA) Primes Eosinophil (EOS) Chemotaxis

J. B. Sedgwick, E. A. Durocher, A. M. Brooks; Department of Medicine, University of Wisconsin - Madison, Madison, WI.

RATIONALE: EOS are the major leukocyte recruited to the airways during loss of asthma control. The mechanism(s) of increased EOS migration is not established, however, uPA has been reported to modulate neutrophil migration. Airway EOS isolated 48h after segmental allergen challenge express increased uPA, which modulates cell adhesion. We hypothesized that uPA also enhances EOS chemotaxis.

METHODS: Peripheral blood EOS were isolated from allergic asthmatic subjects using negative magnetic bead selection and incubated with either buffer or exogenous uPA for 30min at 37°C. Cell-bound uPA was measured by flow cytometry; EOS chemotaxis was determined with 5μm-pore Transwell plates.

RESULTS: Incubation of EOS with exogenous uPA significantly increased cell-bound uPA expression (MFU: 24.1±4.7 vs. 5.1±0.8, p=0.02; % Positive: 94.0±0.9 vs. 51.1±6.4, p=0.005; n=4). Pretreatment with exogenous uPA slightly, but significantly, enhanced EOS chemokinesis compared to untreated cells (% Migration: 2.4±0.7 vs. 1.7±0.5, p=0.03, n=9). Pre-incubation with exogenous uPA primed EOS for increased FMLP- (7.7±1.6% (uPA-treated EOS) vs. 4.9±1.1% (untreated), p=0.02, n=9), PAF- (49.4±4.2% vs. 36.7±3.2%, p=0.007, n=9) and RANTES- (29.5±3.7% vs. 22.7±3.0%, p=0.04, n=8) stimulated chemotaxis. uPA had no effect on eotaxin chemotaxis, nor was it effective as a chemokine when added to the bottom chamber alone or in combination with any of the chemokines.

CONCLUSIONS: uPA selectively primes blood EOS chemotaxis and may promote EOS migration to the airway in asthma. *Funded by NIH grants.*

Funding: NIH