SUNDAY

493 Microbiome of the Lower Airways Alters Corticosteroid Responsiveness in Asthma

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RATIONALE: There have been no functional studies defining the effects of airway microbiome on corticosteroid response in asthma.

METHODS: Bacterial 16s rRNA gene sequencing from bronchoalveolar lavage (BAL) samples of 29 corticosteroid resistant (CR), 10 corticosteroid sensitive (CS) asthmatics and 12 healthy controls was performed. Patients were defined as CR if <10% improvement in lung function following one week of oral prednisone treatment occurred. BAL macrophages/peripheral blood monocytes from asthmatics were stimulated with pathogenic vs commensal organisms, treated with 10^{-6}M dexamethasone (DEX) for 3hr and analyzed for the expression of corticosteroid-regulated genes by real-time PCR. Cellular MAPK activation was assessed by Western blot.

RESULTS: 34 out of 39 asthmatics had expansions of specific groups of microorganisms (>5% of 16s rRNA sequences, >2 fold above microbes present in healthy controls or unique organisms) and significant reduction of sequences for Genera *Prevotella,* major airway commensal organism (23.8% vs 53.2% of total 16s rRNA sequences, p=0.001). Six CR, but none of CS, asthmatics had expansions of *Neisseria*/*Haemophilus*. Preincubation of monocytes and macrophages from asthmatics with *Haemophilus parainfluenzae*, representative pathogenic organism from CR asthma airways, but not *Prevotella melaninogenica,* resulted in MAPK activation, IL-8 upregulation and inhibition of cellular response to corticosteroids (IL-8 fold suppression by DEX 2.5±1.1 vs 14.3±1.3, respectively, p=0.001). Toll-like receptor pathway inhibitors restored cellular sensitivity to corticosteroids.

CONCLUSIONS: A subgroup of CR asthmatics demonstrates lower airway expansion of *Neisseria*/*Haemophilus*, which trigger MAPK activation and inhibit airway macrophages/monocytes response to corticosteroids. Toll-like receptor pathway inhibitors restore cellular sensitivity to corticosteroids in presence of these bacteria.

494 The Importance of Hyaluronan As a Contributor to Asthma Progression

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RATIONALE: Lung extracellular matrix (ECM) hyaluronan produced by fibroblasts is pro-inflammatory, inducing cytokine secretion and leukocyte retention. TGFβ-1 induces differentiation of lung fibroblasts to myofibroblasts which secrete more hyaluronan, contributing to airway inflammation and remodeling. In an animal model of asthma, CD44, the hyaluronan receptor, was required for hyaluronan turnover and clearance of eosinophils. We further evaluated the role of CD44 in hyaluranon-related lung inflammatory processes.

METHODS: Lung fibroblasts were treated with +/-TGFβ-1. A scratch-wound culture was used. In previous studies, injury induced hyaluronan production and hyaluronan addition increased cell migration. Cultures were incubated with fluorescent (hyaluronan (fHA), hyaluronan-binding-protein or antibodies to α-smooth muscle actin (αSMA) or CD44. Eosinophils were fluorescently labeled.

RESULTS: fHA binding sites on migrating myofibroblasts were primarily along cell margins and the ruffling membrane, and did not correspond to CD44 staining. Hyaluronan staining at wound edge and on migrating cells was upregulated. Hyaluronan-rich cell coats were larger and matrix more cable-like on migrating myofibroblasts. CD44 staining, uniform across the luminal surface, was not altered by scratch-wounding. αSMA staining in response to TGFβ-1, was not changed by CD44 knock-out, despite increased hyaluronan accumulation by myofibroblasts. Hyaluronan-dependent eosinophil retention was increased in ECM induced by TGFβ-1.

CONCLUSIONS: Hyaluronan binding and accumulation increased on the forward surface of migrating cells. There was increased hyaluronan staining at the wound edge and TGFβ-1 stimulation increased hyaluronan-dependent eosinophil retention. These processes did not correspond to CD44 distribution. Hyaluronan appears to be important in pulmonary inflammation and may be regulated in a CD44-independent manner.

495 Novel IL33 Gene Polymorphisms Associated with Asthma Are Associated with Resistance to Schistosoma Mansoni

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RATIONALE: IL33 is one of the most consistently associated candidate genes for asthma identified by GWAS in diverse ethnic groups. We previously demonstrated GWAS SNPs associated with asthma were also associated with asthma and schistosomiasis in African-ancestry Brazilians (356 nuclear families) living in Conde, Bahia, a region endemic for Schistosoma mansoni. We expanded these studies to include an additional set of variants selected from a recently completed deep-resequencing study in an African American asthma cohort.

METHODS: Targeted resequencing in 77 kb around the IL33 gene in 183 asthmatic cases and 192 controls yielded 9 SNPs associated with asthma, these were genotyped in 772 Brazilian samples using TaqMan. Hardy-Weinberg testing was performed using PLINK and pairwise linkage disequilibrium was estimated using Haploview. Genetic association tests were performed using generalized estimating equation under a dominant model on the soluble adult worm antigen (SWAP)-specific IgE:IgG4 ratio (a measure of S. mansoniresistance) adjusting for age, gender, water contact index, and African admixture.

RESULTS: Three SNPs were associated with higher SWAP-specific IgE:IgG4 (P=0.001, 0.004, 0.020, respectively). The most significant SNP mapped to intron 1, and the allele conferring asthma risk in the African American cohort also conferred protection against schistosomiasis. This intronic variant is independent from the previously reported GWAS co-associations (R2=0.133).

CONCLUSIONS: Additional genotyping of IL33 variants identified by targeted sequencing demonstrated a novel locus that co-associates with both asthma and schistosomiasis in populations of African ancestry.