Protein Networks Mediating Airway Hyper-responsiveness in Equine Airways

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GOAL: Delineate molecular pathways that direct airway hyper-responsiveness (AHR) in horses as a means to advance knowledge for diagnosis, treatment and genetic manipulation of AHR in diverse species including man

Rationale

- AHR is the keystone of asthmatic bronchoconstriction that drives novel therapeutic development.[1,2] yet the processes that direct AHR are incompletely characterized. Airway smooth muscle (ASM) is a pivotal cell type mediating AHR in human asthma.[3,4]
- AHR is a pervasive characteristic of airway diseases (Recurrent airway obstruction, Inflammatory airway disease, Exercise induced pulmonary hemorrhage) that account for up to 80% of poor performance in horses.[5-10] AHR is also a sequela to viral respiratory infections in other species.[11-14]
- Seasonal exacerbation/remission that characterizes pasture associated Recurrent Airway Obstruction, termed Summer Pasture Associated Recurrent Airway Obstruction (SPARAO),[15] provides a unique opportunity to identify genes that connect AHR and exacerbations of asthma-like disease.

Method

n=6 horses with pasture associated RAO

n=6 age and sex matched control horses co-housed on pasture

Aim #1: Correlate disease severity & airway muscle mass to magnitude of AHR in diseased/ control horses

Aim #2: Identify disease associated differentially expressed gene products and their relationship to AHR

Aim #3: Confirm biologic relevance and cellular location of disease-associated DEGs at the protein level by immunohistochemical localization in lung tissues from a second disease cohort

Hypothesis: Exacerbations of airway hyper-responsiveness in SPARAO susceptible horses reflect increased activity in protein networks that augment ASM contractility and proliferation.

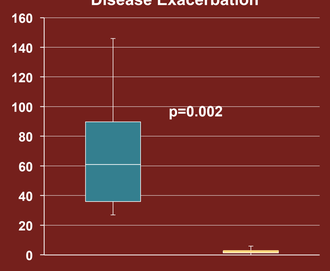
Results:

1. The magnitude of AHR in horses with SPARAO mirrors severe asthma[16]

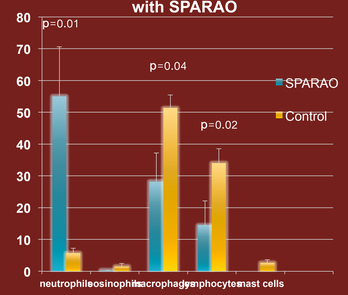
Methacholine Concentrations Causing 40% Increase in R_L								
mg/ml	0.125	0.25	0.50	1	2	4	8	16
# Diseased Horses	2	2	1	1	---	---	---	---
# Control Horses	---	---	---	---	---	---	---	16

2. Neutrophilic inflammation and episodic respiratory distress are significantly increased in horses with AHR

Respiratory Effort[17] Is Increased in Horses with SPARAO During Seasonal Disease Exacerbation



Neutrophilic Airway Inflammation is Significantly Increased in Horses with SPARAO

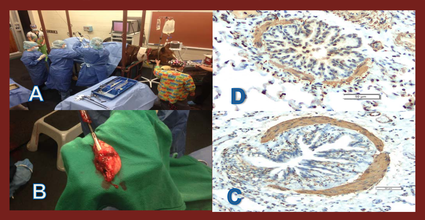


Current analysis:

Histologic sections from lung wedge biopsies are stained with α-smooth muscle actin. Airway smooth muscle is quantified in cross-sectional airways using Image J.[18]

Results:

1. Thorascopic lung biopsies (n=2) attained from disease/ control pairs during disease exacerbation and remission.
2. mRNA sequencing (≥40 million 150bp paired end reads) performed on lung biopsies using strand-specific mRNA libraries (TruSeq, Illumina) prepared from high quality lung total RNA (RIN 8.5-10).



(A) Standing thorascopic surgery for lung wedge biopsy, (B) Lung wedge biopsy derived from the right caudodorsal lung lobe, (C and D) immunostaining for α-smooth muscle actin in small airways from SPARAO affected horse (panel C) and control horse (panel D). Image J will be used to quantify the airway smooth muscle mass with a correction for airway size using basement membrane perimeter.[17]

Current analyses:

1. Identification of differentially expressed gene products (DEGs) by season and disease and genes responding to the magnitude of AHR (methacholine responsiveness) are underway.
2. Canonical pathways analysis will proceed using human orthologs of equine DEGs to identify biological processes, pathways, networks and key genes that are responsive to disease.
3. Over-represented Gene Ontology categories will be identified using GO modeling tools.[19]
4. Biological functions of AHR-associated DEGs will be compared to known protein functions in publich databases that were previously identified in ASM and endobronchial biopsies from asthmatic patients

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Impact

Challenges, Pitfalls and Plans

1) Start date was delayed by needed equine housing improvements. A single horse that required protracted therapy and washout for pneumonia. Sampling for Aim 2 was completed in May 2016.

2) Quantification of airway smooth muscle has been complicated by variability presumably reflecting the difficulty in attaining cross-sectional airways from peripheral wedge biopsies. Re-staining using an alternate chromogen to improve detection is underway. Sectioning technique was modified at remission surgery to increase useable airways. All blocks have been scanned using Aperio® software to enumerate airways. Additional fixed and frozen samples are available. Previous to this project, we developed an alternate scoring system to identify ASM hypertrophy that can be employed (in review Journal of Veterinary Pathology) to assure a potentially important variable in phenotype is included as a factor in transcriptome analysis.

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