Canine Pulmonary Hyalinosis:
An investigation of an idiopathic alveolar filling disorder

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Introduction:

• Rare disease in dogs
  • Incidence unknown
  • Cause unknown

• Disease names:
  • Granulomatous pneumonia with PAS-positive bodies (Billups, 1972)
  • Pulmonary hyalinosis (Dagle, 1976)
  • Surfactant pneumonia (Cooper, 2017)

• Primarily an incidental finding at necropsy
  • Found concurrently with other respiratory diseases
Canine Pulmonary Hyalinosis: State of Understanding

• Alveolar filling disorder
  • Multifocal accumulation of colourless, grey-tan or blue material (H&E stain)
  • Periodic acid-Schiff (PAS)-positive, partial birefringence in polarized light
  • Ultrastructure: lamellar arrays resembling multilamellar vesicles

• Inflammatory response:
  • Macrophage
  • Multinucleated giant cells

• Unknowns:
  • Cause
  • Material identity
  • Material origin

Pulmonary Hyalinosis: Inflammatory cell influx (HE, 200x)
Potential Causes of Pulmonary Hyalinosis:

- Excess endogenous protein production
- Impaired removal of endogenous protein
- Exogenous material + ineffectual clearance

Surfactant

Non-Surfactant

Pulmonary Hyalinosis
Lung Histopathology

Pulmonary Hyalinosis: multifocal, demarcated, amorphous masses (HE, 100x)
Lung Histopathology:

Pulmonary Hyalinosis, HE, 100x

Pulmonary Hyalinosis, HE, 40x
Lung Histopathology:

Pulmonary Hyalinosis, HE, 600x
Lung Histopathology:

Pulmonary Hyalinosis, HE, polarized light, 600x
Lung Histopathology:

Pulmonary Hyalinosis, HE, 1000x

Pulmonary Hyalinosis, HE, polarized light, 1000x
Objective:

To identify the unknown material filling alveoli in canine pulmonary hyalinosis
SDS-PAGE (Polyacrylamide Gel Electrophoresis)

Soluble Fraction

Insoluble Fraction*
Tandem Mass Spectrometry

• Proteomics analysis of insoluble sample
  • Purpose: Identification of distinct proteins

• Four proteins unique to the insoluble fraction in the pulmonary hyalinosis sample

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<tr>
<th>Unambiguos Proteins</th>
<th>Ambiguous Proteins</th>
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<tr>
<td>Pulmonary Surfactant-associated</td>
<td>Uncharacterized protein</td>
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<td>Protein A*</td>
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<td>Uncharacterized protein</td>
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Proteins identified uniquely within the insoluble fraction, based on the criteria: i) 1% false-detection rate to minimize the amount of false positives, (ii) the minimum number of peptides per identified protein specified to 2 and (iii) a set peptide threshold of 95% (indicating the minimum certainty of protein identity must be at least 95%).
Creation of a Tissue Microarray

• 39 cores: eight cases of pulmonary hyalinosis (33 cores) and 3 healthy tissue samples (6 cores)
  • Built with 0.6 mm cores with 1.1 mm spacing

• Staining indicated 100% of pulmonary hyalinosis cases stained positive for PAS and Alcian blue
  • Control cases were unreactive to these stains

Variability in staining of pulmonary hyalinosis bodies with Periodic acid-Schiff
Immunohistochemistry

- Immunohistochemistry for the uniquely identified protein, pulmonary surfactant protein-A
  - JA Ramos-Vara, Purdue University
  - Primary antibody: sc-7700 (Santa Cruz), affinity-purified goat polyclonal antibody to N-19 peptide at N-terminus of human SP-A

- Consistent pattern labeling of material with negligible background staining
Immunohistochemistry for SP-A, 400x
Immunohistochemistry for SP-A, 400x
Summary

• Findings:

1. Variability of intra-alveolar material with respect to HE and PAS staining, and birefringence under polarized light

2. Surfactant protein A is the major identified protein present in pulmonary hyalinosis vs normal lung (n=1 case each)

3. Surfactant protein A is localized to the periphery of the intra-alveolar material based on IHC
Summary

• Implications for pathogenesis:

  • Inhaled/foreign or abnormal endogenous material, with adsorption of SP-A to the surface?

  • Alternatively, abnormal accumulation of SP-A ± surfactant lipids; modification of the material over time may affect its immunoreactivity

• Comparison to pulmonary alveolar proteinosis (different morphology, but perhaps a similar dysregulation of surfactant lipid or protein metabolism)
Acknowledgements & References

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