CHEST

Original Research

DIFFUSE LUNG DISEASE

Chronic Pleuropulmonary Fibrosis and Elastosis of Aged Donkeys

Similarities to Human Pleuroparenchymal Fibroelastosis

Amy Miele, BVM&S; Kevin Dhaliwal, MBChB, PhD; Nicole Du Toit, BVSc, PhD; John T. Murchison, MBChB, PhD; Catharine Dhaliwal, MBChB, PhD; Harriet Brooks, BVetMed, PhD; Sionagh H. Smith, BVMS, PhD; Nik Hirani, MBChB, PhD; Tobias Schwarz, DrMedVet; Chris Haslett, MBChB; William A. Wallace, MBChB, PhD; and Bruce C. McGorum, BVM&S, PhD

Background: **Donkey pulmonary fibrosis (DPF) is a spontaneous syndrome of aged donkeys with a high prevalence (35%). No previous detailed characterization of DPF has been performed. We sought to determine the similarities between DPF and recognized patterns of human pulmonary fibrosis.**

Methods: **Whole lungs were collected from 32 aged donkeys at routine necropsy. Gross examination revealed pulmonary fibrosis in 19 donkeys (DPF cases), whereas 13 (control cases) had grossly normal lungs. Eighteen whole inflated ex vivo lungs (11 DPF cases, seven control cases) were imaged with high-resolution CT (HRCT) scan, whereas the remainder were sectioned and photographed. Tissue samples were collected from all lungs for histopathologic evaluation using a standardized protocol. HRCT images and histology sections underwent independent blinded review. Lung tissue was analyzed for herpes virus, fungal hyphae, mycobacteria, and dust content.**

Results: **Ten of 19 DPF lungs were categorized as being consistent with pleuroparenchymal fibroelastosis (PPFE) according to previously defined histologic and imaging criteria. All 10 PPFE-like lungs had marked pleural and subpleural fibrosis, predominantly within the upper lung zone, with accompanying intraalveolar fibrosis and elastosis. Asinine herpesvirus was ubiquitously expressed within control and DPF lung tissue. No other etiologic agents were identified.**

Conclusions: **Many cases of DPF share key pathologic and imaging features with human PPFE, a rare interstitial pneumonia. Consequently, further study of DPF may help to elucidate the etiopathogenesis of human PPFE.**

CHEST 2014; 145(6):1325–1332

Abbreviations: AsHV = asinine herpesvirus; DPF = donkey pulmonary fibrosis; EVG = elastic Van Gieson; HRCT = high-resolution CT; PCR = polymerase chain reaction; PPFE = pleuroparenchymal fibroelastosis

Pulmonary fibrosis represents the end point of many diseases and is characterized by excessive and irreversible deposition of extracellular matrix in the lung parenchyma, leading to compromised ventilation and organ dysfunction. Despite considerable research, many fibrotic lung diseases remain elusive in terms of etiology, pathogenesis, and treatment.[1] Progress is hindered by the lack of a translatable animal model with durable and persistent fibrosis.[2]

The term "idiopathic pleuroparenchymal fibroelastosis" was coined by the authors of a case study in 2004 to describe a novel clinicopathologic entity that did not fall within the 2002 American Thoracic Society consensus classification of idiopathic interstitial pneumonias.[3] The authors described a predominantly upper zone distribution of pleural and subpleural fibrosis with elastosis and proposed that previous reports of idiopathic pulmonary fibrosis of upper lung lobes were consistent with pleuroparenchymal fibroelastosis (PPFE).[3] PPFE was subsequently included in the 2013 American Thoracic Society/European Respiratory Society statement "Update of the International Multidisciplinary Classification of the Idiopathic Interstitial Pneumonias" in the category of rare idiopathic interstitial pneumonias.[4] Although PPFE is regarded as usually idiopathic, it has been linked with connective

tissue diseases, genetic predisposition, and autoimmunity following organ transplant.[3-5] Reddy et al[5] suggested that repeated inflammatory damage following recurrent infections in predisposed individuals could lead to PPFE and that airway-centered injury could be key to disease pathogenesis.

Donkey pulmonary fibrosis (DPF) is a syndrome that is also sparsely documented, yet a prevalence of 35% at routine necropsy was reported in a UK cohort.[6] Very little is known about this chronic, potentially debilitating, and currently untreatable idiopathic condition. To test our hypothesis that many cases of DPF share the key pathologic and imaging characteristics of PPFE, we performed the most comprehensive systematic characterization of DPF to date.

Materials and Methods

Tissue Collection and Processing

Whole lungs were collected from 32 aged donkeys during routine necropsy at two UK donkey sanctuaries between June 2009 and January 2013 (e-Appendix 1). Nineteen DPF lungs were selected because of grossly visible fibrosis, whereas 13 grossly unaffected control lungs were selected at random. All lungs were manually inflated, the tracheas clamped, and gross images photographed. Tissue samples were collected from each lung into 10% buffered formalin, essentially as described previously,[7] before undergoing routine processing to paraffin blocks. Sections were stained with hematoxylin and eosin, elastic Van Gieson (EVG), and Masson's trichrome. Tissue samples from eight lungs (four DPF, four control) were collected into RNA*later* (QIAGEN) for DNA extraction and subsequent polymerase chain reaction (PCR). Because this study used only ex vivo tissue collected at routine necropsy, licensing on ethical and humane grounds was not required.

Histology

Histology sections were reviewed independently and blindly by three medical and veterinary pathologists with experience in lung disease. Subsequent to the recognition that the changes observed resembled those of human PPFE, the histologic features were categorized as being consistent with or inconsistent with PPFE according to criteria described by Reddy et al.[5] Cases were categorized as consistent with PPFE on histology if (1) there was pleural thickening with associated subpleural intraalveolar fibrosis and alveolar septal elastosis or (2) intraalveolar fibrosis was present but either not associated with pleural fibrosis, not predominantly subpleural, or not in a dorsal lobe sample. Inconsistent with PPFE was assigned to lungs that lacked these features.

High-Resolution CT Imaging and Digital Photography

Eighteen whole inflated ex vivo lungs (11 DPF, seven control) were imaged with a high-resolution CT (HRCT) scanner (Aquilion One [Toshiba Medical Systems, Toshiba Corp] or Somatom Volume Zoom [Siemens AG]). The remaining lungs (eight DPF, six control) were systematically sectioned transversely and photographed digitally. All images were reviewed independently and blindly by an expert radiologist and were categorized as consistent with or inconsistent with PPFE according to criteria described previously.[5] Cases were categorized as consistent with PPFE if (1) there was pleural thickening with associated subpleural fibrosis predominantly in the dorsal lung or (2) there was dorsal lung pleural thickening and associated subpleural fibrosis, but the distribution of fibrosis was not concentrated in the dorsal lung or coexistent lung disease was evident elsewhere. Inconsistent with PPFE was assigned to lungs that lacked these features. Overall, cases were assigned as PPFE-like only if categorized as consistent with PPFE on both imaging and histology.

PCR for Herpesviral Polymerase

Eight lung samples (four DPF, four control) collected into RNA*later* were processed using an AllPrep DNA/RNA Mini Kit (QIAGEN) according to the manufacturer's instructions. For investigation of the presence of herpesvirus, a region of the herpesvirus DNA polymerase gene was amplified using 100 ng DNA per reaction with two sets of nested degenerate primers as previously described.[8] PCR products were cloned into the TOPO TA vector (Invitrogen by Life Technologies) and sequenced (The GenePool), and BLASTn, version 2.6.2 (National Center for Biotechnology Information) was used to align derived sequences against known herpesvirus sequences.

Special Staining

Lung sections in which there was granulomatous inflammation were stained for acid-fast bacteria using a standard Ziehl-Neelsen stain and for fungal hyphae using Grocott's methenamine silver and periodic acid-Schiff stains.

X-ray Diffraction

Formalin-fixed wet lung tissue samples from four DPF ex vivo lungs were pooled, digested in potassium hydroxide, and prepared for mineral particle analysis under transmission electron microscopy at a magnification of 20,000 and for energy-dispersive x-ray analysis. The mass of dust per gram of dry lung tissue was

Manuscript received June 5, 2013; revision accepted December 28, 2013; originally published Online First March 6, 2014.
Affiliations: From the Medical Research Council Centre for Inflammation Research (Drs Miele, K. Dhaliwal, and Hirani and Prof Haslett), Queen's Medical Research Institute, and Royal (Dick) School of Veterinary Studies and The Roslin Institute (Drs Smith and Schwarz and Prof McGorum), University of Edinburgh, Edinburgh, Scotland; The Donkey Sanctuary (Drs Du Toit and Brooks), Sidmouth, Devon, England; and Department of Radiology (Dr Murchison) and Department of Pathology (Drs C. Dhaliwal and Wallace), New Edinburgh Royal Infirmary, Edinburgh, Scotland.
Drs Miele, K. Dhaliwal, and Wallace and Prof McGorum contributed equally.
Part of this article was presented in abstract form at the British Society of Animal Science Annual Meeting, April 16-17, 2013, Nottingham, England, and at the 7th Joint Meeting of the British Division of the International Academy of Pathology and the Pathological Society of Great Britain & Ireland, June 18-21, 2013, Edinburgh, Scotland.
Funding/Support: This study was funded by the Medical Research Council (MR/J014702/1).
Correspondence to: Bruce C. McGorum, BVM&S, PhD, The Royal (Dick) School of Veterinary Studies and Roslin Institute, University of Edinburgh, Easter Bush, Midlothian, Scotland, EH259RG; e-mail: bruce.mcgorum@ed.ac.uk

DOI: 10.1378/chest.13-1306