P476: Long-term pathology of the naturally-acquired *Pneumocystis* primary infection in rats

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INTRODUCCION

- Pneumocystis* is the most common pulmonary infection in healthy infants, with a peak incidence between 2 and 5 months of age that is not diagnosed.
- Recently, we have shown in an animal model that the primary infection induces lung damage consisting of increased mucus, airway epithelial thickening, perivascular and peribronchial infiltrates, and increased collagen that suggests chronic changes secondary to *Pneumocystis* in immunocompetent rats up to day 75 post-infection.
- Whether the primary infection by *Pneumocystis* induces long-lasting pathology is unknown and may suggest a role of this fungal infection in chronic airway disease. Therefore, we extended our evaluations in the same model until the age of 1 year.

METHODS

- 120 newborn rats kept in HEPA-filtered air containers throughout the experiment were randomized to exposure at birth to rats with *Pneumocystis* pneumonia by co-habitation for 48 hours, and to non-exposure. Non-exposed rats received anti-*Pneumocystis* prophylaxis (TMP/SMZ) for the duration of the experiment.
- 12 rats per group were sacrificed each time under deep anesthesia at 40, 60, 80, 258 and 361 days of age. Half (6) received vascular perfusion with 3% buffered formalin via inferior cava vein, and their fixed lungs sectioned longitudinally separating central from distal airway lung zones for histology determinations according to Figs 2 & 3 using Image Pro-plus software. The other 6 rats were exsanguinated and their lungs removed, placed in RNA-later, and frozen fresh at -80°C until used for *Pneumocystis* and collagen determinations.
- Pneumocystis* was diagnosed using n-PCR with specific primers that amplifying the mtLSUrRNA, and collagen levels were biochemically determined by hydroxyproline method.

Figure 3: (A) (C) Mucus content (% epithelium area positive for Alcian blue-PAS) was assessed in rats at 81, 259 & 361 days of age. (B) (D) Representative images showing the progression of mucus within the epithelium.

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References

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RESULTS

- Pneumocystis*-DNA amplified in 6/6(100%), 6/6(100%), 6/6(100%), 2/6(33%), 1/6(17%) of exposed rats and in none (0%) of non-exposed rats at 40, 60, 81, 258 & 361 days respectively (Table 1)
- Inflammatory cuffs increased significantly from day 81 and from day 60 up to day 361 around bronchi and vessels respectively. Cuffing infiltrates were more prominent around vessels.(Fig 1)
- The area of airway epithelium occupied by mucins was 18 and 5 times higher on days 281 and 361 post infection respectively, than measurements before day 80 in the *Pneumocystis*-exposed rats (Fig 2)
- Cuffing inflammation and the area of epithelium occupied by mucins remained stable in the non-exposed rats (Fig 2)
- Collagen levels were not different among groups throughout the experiment.

Age (Days)	<i>P. carinii</i> -DNA Pos./Total (%)
40	6/6 (100%)
61	6/6 (100%)
81	6/6 (100%)
259	2/6 (33,3%)
361	1/6 (16,7%)

Table 1. Detection of *P. carinii*

Figure 2: Percent of airways and vessels with peribronchiolar or perivascular inflammatory cuffing in rats at 40, 61, 81, 259 & 361 days of age. Using microscopy and hematoxylin-eosin stain. Airways (A & B) and Vessels (C & D).

CONCLUSION

- The primary infection by *Pneumocystis* induces long-term changes consisting of persisting peribronchial and perivascular inflammation and severely increased mucin secretion that is consistent with long-term epithelium changes of mucous cells.
- These changes support a role of *Pneumocystis* in chronic airway disease.