Expression of Surfactant Protein-A (SP-A) in the Developing Murine Intestinal Tract

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Background

•SP-A is an important part of the innate defense system with well characterized effects in the lung
•SP-A -/- newborn mice show abnormal bacterial colonization patterns that persist into adulthood, indicating a role for SP-A in the intestine
•SP-A mRNA has been detected in the GI tract, and SP-A protein has been detected in amniotic fluid
•There is controversy in the literature regarding SP-A intestinal expression: many have shown the presence of gene expression, but there is a corresponding lack of protein expression
•It is unclear if newborn exposure to intestinal SP-A is from ingested amniotic fluid or intestinal SP-A production

Methods

•**Tissue Collection:** Tissue was collected from wild-type (C3He/BFeJ) and SP-A -/-mice at PND 3 -7 and flash frozen in liquid nitrogen.
•**Immunohistochemistry:** Tissue was sectioned at 5μm, fixed in acetone, and probed with a Vectastain Rabbit IgG kit. The antibody used was an anti-SP-A rabbit polyclonal IgG, used at a dilution of 1:100 (intestine) or 1:250 (lung).
•**RNA *in situ* hybridization: Probe Synthesis**

Total RNA isolated → Reverse transcribed to cDNA → Amplified via PCR → Transformed into E. coli → Vector isolated and linearized → Transcribed to DIG-labeled RNA

•**RNA *in situ* hybridization**: Tissue was cut at 4 μm, fixed in 3% paraformaldehyde, and hybridized with an antisense or sense SP-A and cμ probe overnight. An anti-DIG antibody (Roche) was used at a 1:500 dilution and detected via NBT/BCIP with levamisole.

Results

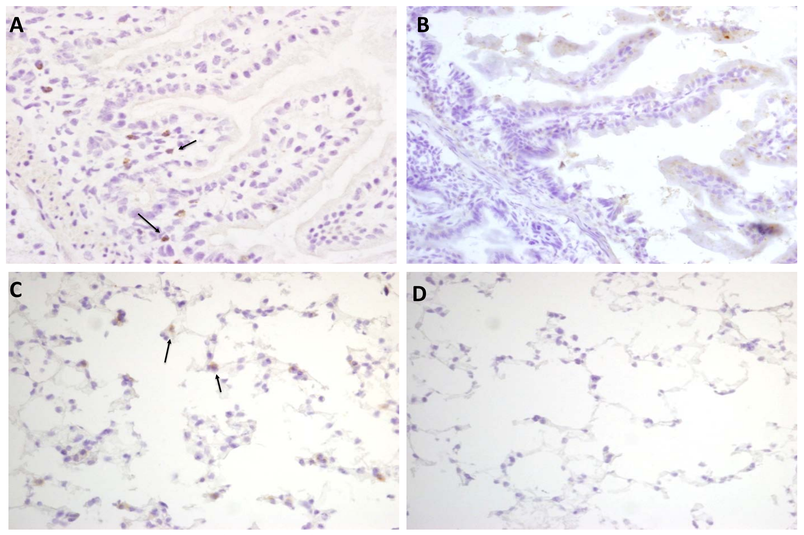

Figure 1. Immunohistochemistry for SP-A. **A.** SP-A staining in a PND 7 wild-type mouse intestinal tract. Stained cells were seen in the lamina propria. **B**. SP-A staining in an SP-A null mouse intestinal tract. Only background staining in visible. **C**. SP-A staining in wild-type adult lung tissue (positive control). Alveolar cells (type II pneumocytes) stained positively. **D.** SP-A staining in an adult wild-type lung without addition of SP-A antibody (negative control). No staining occurred in the negative control.

Supporting Background Data

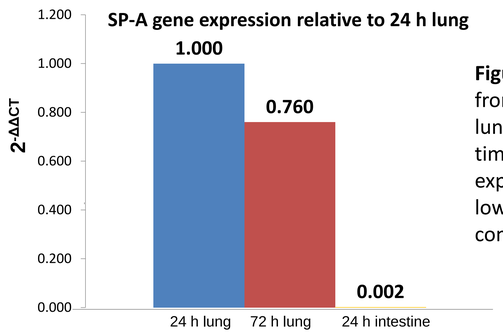

Figure 2. SP-A gene expression from RT-PCR relative to 24 h lung. Expression decreased over time in the lung. SP-A gene expression was significantly lower in 24 hour intestine compared to 24 hour lung.

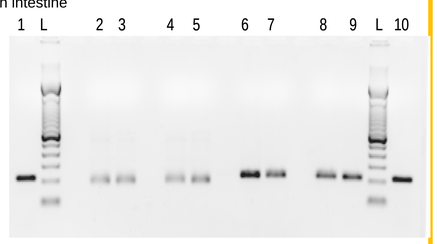

Figure 3. RT-PCR demonstrating house keeping gene GAPDH and the gene of interest, SP-A. Whole intestines from two 14 day old mice (lanes 2-9) and lung (lanes 1 and 10) are demonstrated. cDNA GAPDH expression obtained from 3mcg RNA (lanes 2,3) and 2 mcg RNA (lanes 4,5) demonstrated equal loading. cDNA SP-A expression in the intestines from 3 mcg RNA (lanes 6,7) and 2 mcg RNA (lanes 8,9) migrated to 212 base pairs, the same migration as in the lung (lanes 1 and 10). 2 kB ladders are indicated with an "L".

RNA ISH Progress

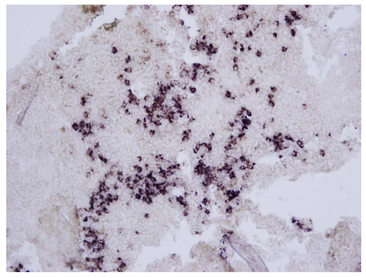

Figure 4. RNA *in situ* hybridization on adult wild-type spleen with cμ probe. cμ serves as a positive control for the *in situ* protocol.

Future work will focus on obtaining similar results with an anti-sense SP-A probe in newborn lung and intestinal tissue

Conclusions

•There is protein expression of SP-A in the lamina propria cells of the GI tract, which was an unexpected location
•Positive staining in IHC corresponds to RT-PCR data of gene expression
•RNA *in situ* hybridization is being used to see which cells are responsible for gene expression and if they match positively stained cells from IHC
•The RNA *in situ* hybridization protocol works, but refinement specifically for SP-A detection is necessary

Acknowledgements

Thank you to Angela Panoskaltsis-Mortari, PhD and Andy Price for the cμ probe and guidance throughout the RNA *in situ* hybridization procedure

References

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