Newborn Mouse Type I Alveolar Epithelial Cells Produce Increased Chemokines Compared To Adult Cells In The First 24 Hours In Culture.

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INTRODUCTION

The alveolar surface is covered by large flat Type I cells (AEC1). The normal physiological function of AEC1s involves gas exchange, based on their location in approximation to the capillary endothelium and their thinness, and in ion and water flux, as shown by the presence of solute active transport proteins, water channels, and impermeable tight junctions between cells. In response to injury, studies from forty years ago indicated that AEC1 cells were derived from Type II cells. It was also felt that AEC1s were terminally differentiated. More recent work on freshly isolated AEC1 cells indicates that they have the ability to produce inflammatory mediators when stimulated *in vitro* and so may have a role in the distal lung innate immune response.

Bronchopulmonary dysplasia (BPD) is a chronic pulmonary condition that occurs in premature infants after exposure to environmental stimuli such as maternal chorioamnionitis, postnatal hyperoxia and/or mechanical ventilation. Since AEC1 are present in the saccular period, when the BPD risk is highest, have their development affected by the simplification of lung architecture which is the hallmark of BPD and since inflammation is a prominent feature and marker of early BPD, we have begun to investigate whether AEC1 cells may play a role in the development of BPD.

OBJECTIVES

- To isolate AEC1 cells from newborn (<24 hours old) and adult mice
- To compare acute *in vitro* chemokine production between newborn and adult mice

METHODS

AEC1 Isolation

- All protocols were approved by the institutional IACUC.
- Newborn = < 24 hours old, adult - > 3 months old. All were C57BL6 mice.
- Lungs from 3 adult mice or a litter of newborns were pooled.
- For adults: - Blood was flushed from the pulmonary circulation by infusing 10-20 ml saline into the right ventricle after incising the left atrium.
  - Trachea was cannulated and lavaged sequentially with 0.05M EDTA/0.05M EGTA, then RPMI1640. Lungs were then inflated with RPMI 1640/4.5 U/ml Elastase/0.1 g/ml Dextran.
- For newborns: - Blood was passively drained from the lungs by removal of the heart.
  - Lungs were removed without tracheal cannulation as above
- Whole adult lung or newborn lungs were incubated for 40 mins at 37ºC in the elastase solution. Airways were dissected away and the lungs minced into fine pieces, then incubated for 15 mins with agitation in 2 mg/ml DNase in RPMI1640
- The material was passed through 100 μm, 40 μm and 20 μm filters, then incubated for 1 hour at 37ºC on petri dishes precoated with mouse IgG. Non-adherent cells were then harvested

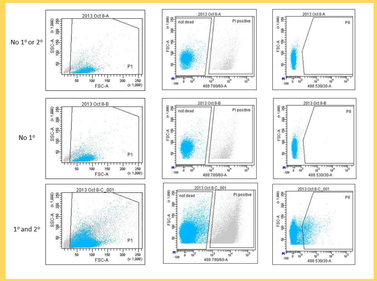
FACS Sorting

- All cells were incubated with Fc block for 10 minutes on ice
- Cells were then incubated with 5 μl hamster anti-mouse T1α for one hour at room temperature, followed by 2.5 μl Alexa 488-labeled anti-hamster IgG.
- Controls received no primary or secondary or just secondary antibody.
- Cells were incubated with Propodium iodide (PI).
- PI negative/ T1α positive cells were collected in PBS/20% FBS

Cell culture

- 19,000 cells per well were cultured on fibronectin-coated Transwells. Apical side contained PBS/20% FBS, basal side contained DME-H16 with 20% FBS and antibiotics in 10% CO_2.

RESULTS



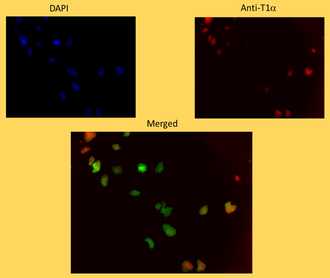


Figure 1 (Left) – Gating graphs from FACS sorting. Top row are cells with no added antibody. Middle row are cells incubated with Alexa-488 2º antibody. Bottom row are cells incubated with both 1º anti-T1α and 2º antibodies.

Figure 2 (Right) - Example of cytospin immunohistochemistry. Cells were stained with T1α primary and Alexa 488-conjugated goat anti-hamster IgG and counterstained with DAPI 0.1 μg/ml. Pseudocolor conversion of anti-T1α slide.

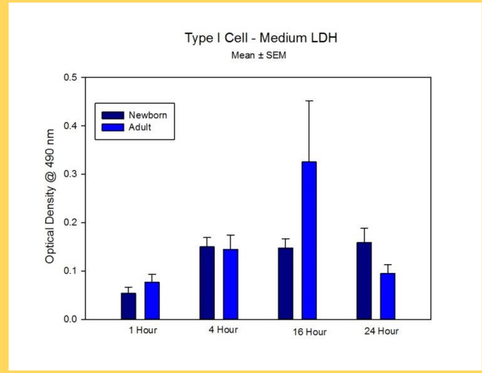
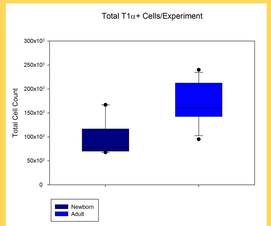


Figure 6. LDH levels in medium in first 24 hours after isolation. There were no differences within age groups over time or between groups at any time point. Two-way ANOVA, p = 0.887



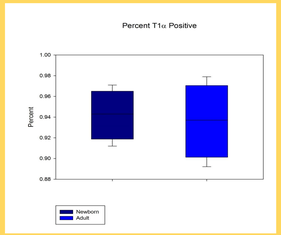


Figure 3 (Left) – Box-and-whisker plot of number of T1α + cells collected in each experiment (3 adult or 1 litter newborn). Median and 25th-75th percentile box, 10th and 90th error bars, outlier dots. N = 10 for adult, n = 8 for newborn. There was a significant difference between groups by t-test, p = 0.0011

Figure 4 (Right) Box-and-whisker plot of percent of DAPI+ cells also positive for T1α by immunohistochemistry. Median and 25th-75th percentile box, 10th and 90th error bars. At least 100 cells/experiment. N = 4 for adult, n = 3 for newborn. There was no difference between groups by t-test, p = 0.7921

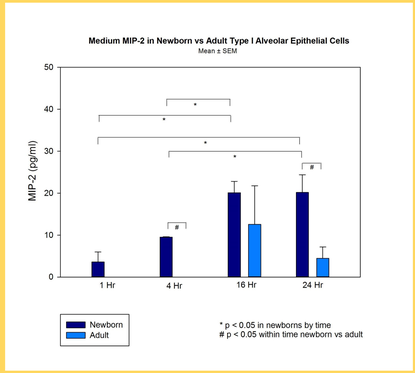


Figure 5. MIP-2 levels in medium in first 24 hrs after isolation. Newborn levels rose significantly by 16 and 24 hrs over 1 and 4 hrs (*), p < 0.05. The newborn levels were higher than the adult levels at 4 and 24 hours (#), p < 0.05 by Student-Newman-Keuls analysis.

CONCLUSIONS

- AEC1 cells can be isolated from both adult and newborn mice
- Purity is 90% or more and averages 94%
- AEC1 cells from newborn mice cultured in for 24 hours produce higher amounts of the chemokine MIP-2 than cells from adult mice
- This increase is not due to increased cell death, as shown by the lack of any difference in LDH levels

SPECULATION AND FUTURE DIRECTIONS

- AEC1 cells can be isolated from mice, including newborn mice
- Overall cell yield is low and needs to be improved
- If confirmed, AEC1 cells may be additional source of inflammation in early stages of BPD
- Not clear if the increased in chemokine from newborn AEC1 is from new synthesis or from release of preformed MIP-2
  - No evidence that normal newborn mouse lung has increased stores of MIP-2
  - No increase in LDH as measure of loss of cell membrane integrity
- Will need to complete analysis of MIP-2 mRNA by qPCR to determine if this is *de novo* synthesis
- If confirmed, will need to determine mechanism. AEC1 cells express high levels of RAGE, which can turn on MIP-2 expression when engaged.
- If yield can be increased, further studies of behavioral and developmental differences between newborn and more mature AEC1 may provide valuables insights into normal and disordered development

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