Human amnion epithelial cells alter lung development and inflammation in 7-day-old preterm lambs exposed to inflammation before birth

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Background: Lung inflammation and impaired alveolarisation are hallmarks of bronchopulmonary dysplasia. We hypothesized that postnatal treatment with human amnion epithelial cells (hAECs) would reduce lung inflammation and change lung structure, in preterm lambs born after antenatal exposure to inflammation.

Method: Pregnant ewes received either intra-amniotic (IA) lipopolysaccharide (LPS, from *E.coli* 055:B5; 4mg) or saline (control: n=8) on day 126 of gestation, followed by antenatal betamethasone (5.7 mg) 48 h prior to delivery. Ewes received general anaesthesia by sodium thiopentane (~20mL IV) prior to intubation and anaesthesia was maintained (2-3% isoflurane in oxygen). Lambs were delivered by caesarean section at 128 d gestation (term ~150 d). Preterm newborn lambs were managed with graded de-escalation of respiratory support aimed at early weaning from mechanical ventilation to eventual unassisted breathing of room air. LPS-exposed lambs received either IV hAECs (LPS+hAEC, n=7; 10×10^6 cells/kg) or saline (LPS+Sal: n=10) immediately after birth. Lung tissue was collected 1 week after birth. Messenger RNA levels for inflammatory and developmental mediators were measured in lung tissue homogenates using Fluidigm Biomark HD Taqman assays. Tissue and airspace fractions were measured and septal crests counted in sections of lung tissue using unbiased stereology. Data were compared between groups by Kruskal-Wallace ANOVA on ranks and Dunn's multiple comparisons test. Data are expressed as mean ± SD.

Results: Duration of mechanical ventilation was longest in the LPS+hAEC animals than for any other group over the seven day study. The amount of time extubated was highest in the control group. Oxygen requirements were similar between treatment groups. The airspace fraction in the LPS+Sal group (44±3%) was lower (*i.e.* tissue fraction was higher) than in control lambs (52±2%; *P*=0.06); values in LPS+hAEC lambs were intermediate (50±2%). Septal crest density tended to be higher in LPS+hAECs lambs (3.1±0.7%) than LPS+Sal lambs (1.4±0.3%; *P*=0.08). Lung leukocyte and macrophage cell counts were not different between groups. Lung mRNA levels of *IL-1*β and *IL-8* were higher in LPS+Sal and LPS+hAEC lambs than controls (*P*=0.04 and *P*=0.04, respectively). Gene (mRNA) expression of surfactant protein (SP) A and D was increased in LPS+hAEC lambs 7-fold (*P*=0.03) and 15-fold (*P*=0.03), respectively, compared to controls.

Conclusions: Intravenous administration of hAECs may increase ventilator requirements, but has beneficial effects on lung development in preterm lambs exposed to inflammation before birth, in association with activation of the pulmonary immune system.