STRONG CHILDREN'S RESEARCH CENTER

Summer 2015 Research Scholar

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ABSTRACT

Title: ***Neonatal Oxygen Exposure leads to Altered Alveolar Epithelial Type-II cell pools, affecting the Response to Influenza A Virus at 2 week and 4 weeks of Age***

Background: Premature infants, specifically those at low birth weight, often have underdeveloped lungs that are physiologically and structurally immature, making them susceptible to sequelae as developing children and young adults. In addition, children born prematurely often display reduced pulmonary lung function and lung capacity, placing them at increased risk for numerous disorders such as bronchopulmonary dysplasia (BPD), respiratory viral infections and asthma. Such changes have been attributed to life-saving early-life exposure to oxygen that reprograms the development of the lungs, eyes and brain. Oxygen supplementation during the neonatal period influences lung development, alters respiratory abilities, and hinders host defenses of the lung, signifying the need to understand how prematurity and early-life exposure to oxygen affect health later in life.

Our lab has previously demonstrated that neonatal hyperoxia rapidly stimulates expansion of alveolar type II cells compared to mice birthed into room air immediately following neonatal oxygen exposure. Type II cells express innate immune genes, produce surfactant critical for proper gas exchange, and may act as progenitor cells after injury. This expanded population is subsequently depleted during recovery in room air, resulting in an adult lung with proportionally fewer type II cells than those of siblings exposed to room air at birth. Following 8 weeks of recovery in room air, mice exposed to neonatal hyperoxia develop lung simplification as determined by a reduction in the number of pro-surfactant protein C (SP-C)-positive alveolar type II cells.

Design/Methods: To understand how neonatal oxygen reprograms the lung, a mouse model was developed in our laboratory wherein newborn mice are exposed to 21% oxygen (room air), or 100% oxygen (hyperoxia) between postnatal days 0 and 4. Following postnatal day 4, hyperoxia treated mice are returned to room air for recovery. The mice remained in room air for of 2 and 4 weeks. Following recovery litters were infected with Influenza A Virus (IAV); the litters included both male and female pups. Pups were anesthetized with an intraperitoneal injection of Avertin (2,2,2 tribromoethanol; Sigma-Aldrich, Milwaukee, WI). Pups were infected with 20 μl of $5X10^4$ PFU (strain A/HKX31; H3N2) or PBS intranasally. Following infection mice were weighed daily to monitor weight loss. Additionally, control mice were collected prior to

infection for analysis. Manual counting and quantification was conducted using SP-C and ABCA3 staining, two different genes expressed by alveolar type II cells. Western blotting for SP-C was also conducted and electronically quantified.

Hypothesis: Neonatal oxygen exposure leads to a rapid expansion and then pruning of alveolar type II cells. At 2 weeks of age, hyperoxia exposed mice possess a heightened number of type II cells. The ability of type II cells to secrete various antiviral components and act as immune cells subsequently protects mice from respiratory infections such as IAV as 2 weeks of age.

Results

2-Week Model: Prior to infection, a group of room air exposed and hyperoxia exposed mice were collected at 2 weeks of age. Lung sections were stained for the type II cell marker SP-C and positive cells were manually counted and quantified; 10% of room air exposed mice alveolar epithelium was SP-C positive in comparison to the 16% composition in hyperoxia exposed mice. Western blotting for SP-C as well as staining for ABCA3, another type II cell marker, was then conducted. Both experiments indicated a heightened number of alveolar type II cells in hyperoxia exposed mice. As opposed to the control groups infected with PBS, both IAV infected groups, Hyperoxia and Room Air, immediately began to lose weight after infection. By day 10-post infection 100% of the Hyperoxia mice had survived while only 50% of Room Air mice had recovered from infection.

4-Week Model: Prior to infection, a group of room air exposed and hyperoxia exposed mice were collected at 4 weeks of age. SP-C staining was conducted on inflated lung slices and SP-C positive cells were manually counted and quantified; 14% of alveolar epithelial cells were SP-C positive in both the room air exposed and hyperoxia exposed lung samples. Western blotting for SP-C as well as staining for ABCA3 were then conducted, both experiments indicated no significant difference in type II cell numbers between the two groups. Unlike the 2-week study, once infected with IAV both experimental groups lost weight and recovered at relatively the same rate. A greater percentage of mice survived in the 4-week study as opposed to the 2-week study, however the difference in survival was not of any significance.

Conclusions:

Our lab has previously determined at 8 weeks of age hyperoxia exposed mice lose more weight and have a lower rate of survival after IAV infection compared to their room air exposed littermates. This increase in mortality and morbidity can be attributed to the decreased numbers of alveolar type II cells hyperoxia exposed mice possess as young adults. In order to evaluate our hypothesis, histology and immunochemistry analyses were conducted. In the 2-week experiment conducted, 10% of room air exposed mice alveolar epithelium was composed of SP-C positive cells in comparison to hyperoxia exposed mice's 16% composition. After the analyses of the uninfected tissue samples, it was concluded that the increased type-II cell numbers in hyperoxia exposed mice at 2 weeks of age facilitated protection from IAV infection. In the 4-week model, hyperoxia and room air exposed mice possessed relative numbers of SP-C positive cells, indicating

analogous type II cell populations. At this time point morbidity and mortality outcomes due to IAV infection were very similar. These similar outcomes were presumably due to their relative numbers of type II cells, subsequently generating immune responses of comparable magnitudes. Taken together this data suggests that early life oxygen exposure disrupts the proper number of type II cells required to defend the lung against respiratory infection, providing protection at 2 weeks of age and increasing susceptibility as the mice grow into adulthood.